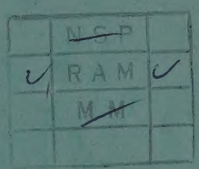
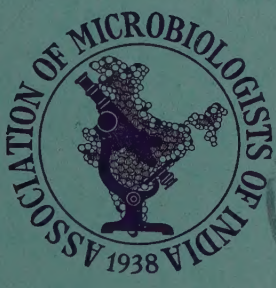
Volume I April—June, 1961 Number 2

INDIAN JOURNAL OF MICROBIOLOGY

Edited for the ASSOCIATION OF MICROBIOLOGISTS OF INDIA





Published quarterly by the
ASSOCIATION OF MICROBIOLOGISTS OF INDIA
P27 PRINSEP STREET, CALCUTTA-13

INDIAN JOURNAL OF MICROBIOLOGY

The Association of Microbiologists of India was founded in 1958 with the object of promoting the study and application of microbiology in India. It provides a common forum for the discussion and evaluation of new knowledge in the different scientific and applied aspects of the study of micro-organisms.

The central office is located at the Indian Institute for Biochemistry and Experimental Medicine, P-27, Prinsep Street, Calcutta-13. All correspondence regarding Association business and managerial matter of the Journal should be addressed to the General Secretary at this address.

The Indian Journal of Microbiology will be published quarterly on the last day of March, June, September and December.

Members of the Association will receive the Journal free. For non-members, the annual subscription is Rs. 15/- (inland) and Rs. 20/- (overseas). Single issues are priced at Rs. 4/- (inland) and Rs. 6/- (overseas).

Fifth Idian Microbiological Congress

Madras—1962.

The fifth Annual Congress and Scientific Session of the Association of Microbiologists of India will be held in the Madras Medical College from 2nd to 4th March, 1962. Apart from business meetings, there will be morning and afternoon sessions for *presentation of original papers and demonstrations.* Those willing to read papers are requested to send the titles and a 250 word summary of their proposed communications to the General Secretary not later than 1st February and the full paper by 15th February, 1962. In addition to reading of papers, *a Group Discussion on the Need for Centres of Type Culture Collection of Micro-organisms in India* and *a symposium on Microbial Variations* will be arranged. Contributors and participants wishing to show slides other than of the standard size ($3\frac{1}{2}'' \times 3\frac{1}{2}''$) should inform the General Secretary of the relevant details when submitting abstracts. A detailed programme of the meeting will be circulated to all members after it is finalized.

It has been decided to organize an *Exhibition of Scientific Instruments and Drugs* by interested firms during the Congress.

An Excursion trip to important places in and around Madras is likely to be arranged for the participants.

S. MUKERJEE
General Secretary

INDIAN
JOURNAL OF
MICROBIOLOGY
Vol. I, No. 2, 1961

Proceedings of the

FOURTH INDIAN MICROBIOLOGICAL CONGRESS

The Fourth Annual Congress and Scientific Session of the Association of Microbiologists of India was held at the Delhi University, Delhi from March 31 to April 2, 1961 under the presidentship of Dr. J. C. Ray. Both from the organizational and scientific points of view the session was widely acclaimed as having set a high standard of accomplishment. Nearly sixty papers were presented, embracing all aspects of the science of microbiology, and the discussions were as lively as they were illuminating. There were besides, a group discussion on the teaching of microbiology in Indian Universities, and a symposium on "Trends in research on viruses and virus diseases in India." For the benefit of the members who were unable to attend, we publish the Proceedings of the Congress and the Session.

Inauguration

The Congress was inaugurated at 9.30 A.M. on March 31, 1961. Welcoming the delegates to the Congress, Dr. R. Viswanathan, Chairman of the Reception Committee

observed that the wide range of papers to be presented at the Congress and their high quality were indication of the growing numbers of microbe-hunters in India. It was essential for the development of any scientific discipline that workers met together for the exchange of ideas at such symposia. Though not a microbiologist himself, he had to work all the time in symbiosis with them, as the study of the morphology and metabolism of microbe parasites formed an important area of medical research. He welcomed the delegates on behalf of the Reception Committee and wished them all good hunting.

Inaugurating the Congress, Professor N.K. Sidhanta observed that though he had no pretensions to being a microbiologist, the Head of a University had to take an intelligent interest in all branches of knowledge. In a brief review of the history of microbiology he referred to classical writers of 2000 years ago, who had prophesied the existence of animalcules not visible to the naked eye. The invention of the microscope by the Dutch scientist Leeuwenhoek, more than 1600 years later stimulated the study of these tiny organisms and established that they possess all the characteristics of life—metabolism, growth and reproduction. Micro-organisms have since been extensively studied as the friends as well as enemies of man. Their study has a recognized position in the work of science now, which is no longer confined to medicine but includes also agriculture and industry. While microbiological knowledge as applied to human activities had thus grown, the universities had remained conservative in giving microbiology the place it deserves as a basic science. In spite of its wide sphere of application, microbiology had undoubtedly become a distinctive branch of science, requiring systematic study. It should occupy a position similar to that of new sciences like statistics and biochemistry which have been given an independent academic existence since the last 30 or 40 years in the Indian universities. He looked forward to the Association of Microbiologists giving a lead to the universities in developing the systematic study of microbiology as a science. He concluded by expressing the hope that the scientific deliberations of this session could prove as fruitful as they had been in the past sessions and that conclusions of far reaching scientific importance would be arrived at under the leadership of Dr. J.C. Ray.

The Secretary of the Reception Committee, Dr. W.V.B. Sundara Rao then read out messages received from the President Dr. Rajendra Prasad, the Prime Minister Shri Nehru, Union Ministers Shri S.K. Patil and Shri Karmarkar and the President of the International Association of Microbiological Societies, Dr. Stuart Mudd, wishing the Conference every success.

Presidential Address

Delivering his presidential address, Dr. J.C. Ray said:

It is with a sense of high privilege that I rise to address the distinguished gathering that has assembled for the inauguration of the Fourth Scientific Congress of our Association. The presence of so many leaders honoured in the public life of our country is gratifying evidence of the support and sympathy that the work of our Association now attracts, as it is an incentive and inspiration to all of us in the Association to persevere in the promotion of microbiological knowledge and its application to national ends.

It should be of special pleasure to all of us in the Association to have General S. S. Sokhey, with us on this occasion. We owe the present stature of our Association to him.

Indeed, he gave something of his own scientific eminence to the Association whose destinies he so signally helped to shape.

We are privileged to have the Congress inaugurated by Professor N.K. Sidhanta Vice-Chancellor of the University of Delhi. He has been very kind to us. As you will remember, he inaugurated the last Scientific Congress of the Association in Calcutta. Since then we have been pursuing him, for the future of microbiology in the country is ultimately in the hands of the universities. It is a guarantee of our hopes for the future that Professor Sidhanta, despite the many preoccupations of his academic leadership, is here in active association with us.

It is a disappointment to all of us that Professor Humayun Kabir could not be present at this morning's function. We would have been heartened in our work by his words of wise counsel and kind encouragement. Knowing however his abiding interest in the advancement of science and the fortunes of scientific bodies, we may feel assured of his blessings and support for our proceedings.

We have a number of distinguished guests. I am particularly happy to see Colonel Felsenfeld, the eminent bacteriologist from the States. We are deeply grateful to him for responding to all our invitations.

With this year's Congress and the publication of the first number of the Indian Journal of Microbiology, our Association has really come of age. Starting over thirty years back, it has been a long and uphill journey to the present day. It is only since 1957, when a far-reaching programme of re-organisation was taken up that the Association has been functioning as a truly All-India body, deriving its membership from workers in all the diverse branches of microbiological science. During the last four years, its growth has been rapid and it has advanced its reputation as a learned body aiming at the integrated advancement of microbiological knowledge and its application in India.

It is hardly necessary at this time of day to argue the need for an Association seeking to bring together in active intellectual cooperation the nation's workers in the field of microbiology. The life stream that feeds progress in the specialised branches of microbiological knowledge derives from the broad scientific basis of microbiology as a distinctive and unified discipline of study. The primary task of our Association, to my thinking, is to promote the free flow and fruitful inter-communication of ideas between the different specialities of microbiological research, and help to form an integrated view of their individual contributions in the perspective of the science of microbiology as a whole.

It will have been remarked that the history of our Association reflects closely the trends of scientific development in India. In the years before independence, the Association struggled on in comparative obscurity and could survive only in a secondary role to medicine and pathology. But even after independence, when scientific and technological development became the main plank of national economic policy, there was a lag in the support given to microbiology, as to the other biological sciences. This was perhaps inevitable in the first stage of industrial development, when the physical and technological sciences had more immediate relevance to the establishment and expansion of primary industries. But I would urge that the stage has now come when the biological sciences should receive equal if not greater attention, important as they are to every facet of national development—public health, industrial productivity, food and agriculture, and population control.

It is perhaps not sufficiently realized that the achievements in the biological sciences during the last fifty years have been as extensive and far-reaching in their implications for human welfare as the more spectacular advancement in subjects like nuclear physics and electronics. As far back as 1948, the number of workers engaged in biological research in the USSR was 100,000 and the figure should have increased considerably since then. Similar importance is attached to fundamental biological research in other scientifically advanced countries also, as may be seen from the vast increase in the world output of original literature in the biological sciences. The reason for this is not far to seek. Results of immediate, and in many cases, of revolutionary significance to practical medicine, agriculture and industry have come from investigations in these fields.

I believe there are already signs of change in our Government's policy of scientific development and we may expect that the biological sciences will receive greater attention in the coming years. Intensive effort is called for, however, as the pace of progress on the expanding frontiers of biology is accelerating all the time, and we have more leeway to make up here than in the physical sciences.

While on the subject of scientific development in India, I should like to stress the role of research journals in raising the level and quality of scientific work. The standards maintained by a country's scientific journals are not only the basis of its scientific reputation, but also condition the quality of originality and intellectual effort which the individual scientist brings to his work. The maintenance of the highest standards of scientific journalism requires, *a priori*, that it be free from the restraints inevitable to commercial operation. The cost of production of technical journals is high, and it would be idle to expect any scientific journal, particularly in a specialised field, to be run on a basis of self-sufficiency. This is a problem we are ourselves facing in regard to the Indian Journal of Microbiology.

Apart from this, there is also unquestionable need to improve the standards of production—of format, paper and typography—of Indian scientific journals. Only by improving their standards of production could our scientists be induced to publish their best work inside the country and thereby raise the level of the scientific content of our journals. I would therefore put in an earnest plea for wider and more generous Government support to our scientific journals.

I would also suggest that scientific meetings and congresses, such as this, should receive greater encouragement. As in every field of human activity, the personal element plays an important part in scientific work also. In a vast country like ours, with the centres of research situated long distances apart, scientific meetings provide the only opportunity for our workers to come together and get to know each other. They also enable informal discussions and the exchange of ideas at a personal level, which are an essential stimulus to scientific activity. The Government should further widen opportunities for such meetings, especially for junior workers, by a more liberal policy in the grant of railway concessions.

We have over 60 scientific papers to be presented and discussed, besides the group review of microbiological teaching in India. I shall not therefore further prolong my prefatory remarks, but pass on to a brief review of immunological theories which I have selected for the subject of my address, "Cellular mechanism of immunogenesis: a review of recent concepts and theories."

The classical picture of immunogenesis, "the ebb, flow and reflow and maintained

high tide'' of the formation of antibodies, would appear to characterize also the history and development of the science of immunology. After a period of intensive activity extending over about 30 years from 1880, when the scientific study of the mechanism of immunity exercised the interest of bacteriologists and chemists alike, interest in the subject declined during the next 30 years. Since the forties of this century, however, interest in the subject has steadily revived and has now reached a peak with the exciting developments that have come from the laboratories of Burnet, Medawar, Kunkel, Grabar and others. A contributory reason to this revival of interest has been the development of biochemical and biophysical techniques that enable studies of the immunity process at the molecular level. Advances in our knowledge of protein chemistry and techniques of characterizing protein molecules have been of particular importance in this respect. The intensification of activity in the biophysical study of proteins holds out promise of further and extensive advances in the field of immunology. It appears timely therefore to review current theories and concepts of immunogenesis, which are having so wide an impact on biological thinking to-day.

The foundations of immunological research were laid by Pasteur's work on chicken cholera, anthrax, swine erysipelas and rabies between 1880 and 1888. Characteristically, Pasteur directed attention to both the practical and theoretical possibilities of the phenomenon of immunity. The success achieved in the direction of practical application has influenced the very course of human history and I shall not concern myself further with this aspect than to remark on the ever-widening field of application of immunological techniques and ideas, which now extends to such apparently disconnected specialities as tissue grafting, enzymology and cancer research.

By the end of the nineteenth century the cardinal experimental facts about immunity had been established and the subject had outgrown the confines of bacteriology. Antibodies were shown to be formed against a variety of chemical substances of non-bacterial origin, including plant proteins like ricin. The specificity of antibody to antigen had been demonstrated and the laws of heredity shown to be inapplicable to the phenomenon of immunity.

Among the earliest theories of the biological basis of immunity was Pasteur's ''exhaustion'' hypothesis, according to which the infecting organism was conceived as using up the entire content of some essential nutrient present in the host's body. This was soon shown to be untenable, as also Chauveau's ''retention'' theory which postulated the retention of an antiseptic substance derived from the infecting organism. The role of the host animal was first emphasized in Metchnikoff's theory which considered immunity to be a cellular process mediated by phagocytosis. Metchnikoff's ideas continued to stimulate experiment and discussion for many years, and have left their imprint on current concepts of immunity.

The theory which gained the widest acceptance, however, was that of Ehrlich, known as the ''side chain'' or ''receptor'' theory. It had its origin in Ehrlich's classical work on the quantitative nature of the toxin-antitoxin reaction. He developed the idea that the lethal action of a toxin and its antitoxin combining power were distinct and separate functions of the toxin molecule. The toxin molecule was assumed to have two different chemical groups, one the 'haptophore' bringing about union with the antitoxin, and the other 'toxophore' responsible for the toxic action. Starting with the idea that the poisoning action of a toxin is the consequence of the specific union of its haptophoric

groups with the 'side chains' of susceptible cells in the host's body, he postulated that the defective cells were stimulated, as the result of a compensatory response, to excessive production of the specific side-chains masked by the formation of the haptophoric complex. The excess of side-chains ultimately cast off into the blood-stream constituted the antitoxin possessing specific affinity for the haptophoric groups of the toxin. Ehrlich later extended this concept to the formation of lytic, agglutinating and other antibodies.

Ehrlich's theory, based as it was on an essentially chemical approach, marks an important change in the direction of immunological thought and research. It anticipated, in essence, the theory of 'natural selection,' so widely held to-day. It also brought home the possibilities of immunological research to the chemist. I may mention particularly the work of Arrhenius and Madsen following up Ehrlich's idea of stoichiometric chemical combination between toxin and antitoxin. They found, however, that the reaction did not proceed in step-wise fashion, as would be expected on Ehrlich's view, but gradually as in the neutralization of a weak acid by a weak base.

Theoretical interest in the molecular mechanism of immunogenesis was stimulated in recent years by Landsteiner's researches on immunological specificity. He showed that a theoretically infinite variety of synthetic organic compounds possess the capacity to elicit specific antibody response. Specificity, which was defined by Landsteiner as "the disproportional action of a number of similar agents on a variety of related substrata" is a fundamental biological property common to enzymes, hormones, nucleic acids, viruses, antibodies and toxins. But antibodies are unique in being produced in response to stimulus by an unlimited range of substances. Landsteiner's work established unequivocally the complementariness of structure between antigen and antibody.

Therefore, when Linus Pauling put forward his theory of antibody formation in 1940, the known facts appeared fully to support the view that antibodies represent a unique modification in the synthesis of natural proteins, corresponding to information supplied by the stimulating antigen. The theory fitted in with the pivotal facts of immunogenesis—the ability of many invertebrates to form specific antibodies to any one of a large number of substances, the specificity of the antibody formed to the antigenic substances administered, and the improbability of an animal possessing of itself the information necessary to synthesise so many different types of antibodies, especially to unbiological organic compounds to be found only in the chemical laboratory.

Pauling's theory was a classical attempt at simplification. He proposed that the antibody has a complementary steric relationship to specific patterns on the surface of the antigen molecule. These complementary patterns had to be laid down against the antigen molecular itself. Pauling postulated accordingly that all antibody molecules contain the same polypeptide chains as normal globulin and differ from it only in the configuration of the chain, that is, the manner of folding of the peptide chains comprising the molecule.

For the understanding of the chemical basis of the antigen-antibody reaction, the theory was eminently satisfying. It still remains the most fruitful approach to the study of the immunity process at the biochemical level. It conforms to the mass of experimental facts accumulated on the combining powers of antibody molecules with haptens related structurally to the immunizing antigen. The combining power can in fact be predicted from the interaction energy of a related hapten for an antibody combining region complementary to the original haptenic group. The theory also accounts for the versatility of the

antibody response, by relating it to the versatility of protein configuration which is an established fact.

Pauling's theory does not however appear adequately to explain certain important biological aspects of antibody formation, particularly the persistence of 'immunological memory.' Though the persistence of a few antigen molecules, necessary according to Pauling's theory for the continued formation of antibody molecules, cannot be ruled out on the existing evidence, the kinetics of antibody formation in relation to the number of available antigen molecules presents a real difficulty. It has been calculated that the number of antibody molecules produced in 48 hours may be 100,000 times as many as the maximum number of antigen molecules that may be left in the entire body. This involves the improbably high rate of the direct antigentemplate producing one molecule of antibody per antigen molecule per second. The theory does not fit in also with the phenomenon of anamnestic response and the exponential rise in antibody production following a second injection of antigen.

Taking account of these and other discrepancies from observed data, Burnet and Fenner put forward an alternative theory in 1949, which they later modified to some extent in 1956. They proposed that the antigen brings about a modification of the enzymes responsible for globulin synthesis and the enzymes are thereby induced to produce a new globulin complementary to the inducing antigen. They postulated also that the antibody-producing cell has some means of recognizing 'self' from 'non-self' material and that the capacity for antibody production can be passed on to descendant cells. Their theory was largely influenced by the analogy, which they emphasized, of antibody formation to the induction of adaptive enzymes in bacteria.

It is pertinent at this stage to consider the salient features of the phenomenon of induced enzyme formation. As far back as 1900, Dienert had pointed out the close resemblance between enzymic adaptation in bacteria and antibody formation in mammals, both adaptive processes of the organism to specific changes in environment. It is now established that enzyme induction does not result in a new pattern of protein structure in the cell. It only involves an increased rate of formation of an enzyme that the cell is genetically capable of producing and, in fact, does produce to some extent before exposure to the inducer. The quantity of enzyme induced is not related to the amount of functionally active inducer and is approximately uniform in the individual cells of a genetically homogeneous population. It has also been proved recently that enzyme induction involves the synthesis of the enzyme protein from its constituent amino acids.

It has thus become apparent, since the formulation of Burnet's theory, that the process of enzyme adaptation does not involve any instruction given to the cell, which it does not possess of itself. It has in fact been shown that different inducers may induce exactly the same protein molecule, as judged by its chemical, enzymic and immunological properties. Further, inducible systems have been found capable of changing to constitutive ones as a result of mutation. The mutated systems form the particular enzyme without external stimulus without having acquired the capacity to synthesize the inducer endogenously. The constitutive enzyme has exactly the same physical and chemical properties as the inducible enzyme.

With the growing evidence that enzyme induction does not involve an instructive process and consists only in the release of a genetically inherent capacity of the cell itself, the analogy between enzyme induction and antibody synthesis, so fruitfully developed

by Burnet, was considerably weakened. Further, electrophoretic studies had revealed that antibodies may be found in different globulin fractions. More recently, it has been shown that even within the gamma-globulin, a single antigen may lead to the formation of different antibody molecules which can be distinguished, for example, by their different degrees of affinity for the hapten. This is indeed weighty objection to both the direct and the indirect antigentemplate theories. These difficulties are satisfactorily resolved in the 'natural selection' theory propounded by Jerne in 1955.

The immediate origin of Jerne's theory was in his observation that normal horse serum contains small amounts of a globulin which stabilizes tryptophan activation of tryptophan-dependent strains of phage T4. In the earliest stage of immunization of a horse with this phage, the particular globulin increases 100 to 1000 fold. Despite the fact that it has no inactivating action on the phage, Jerne considered it to be a naturally occurring antibody, whose production was stepped up by antigenic stimulus. Jerne proposed therefore that a specific antibody conforms to pre-existing protein patterns which may be present in undetectable concentration among the globulins of normal serum. The action of the antigen molecule is to select out, for differential proliferation, such pre-existing patterns as possess a complementary steric relationship to the antigen molecule.

The 'natural selection' theory has received considerable support. It explains satisfactorily the heterogeneity of the antibodies combining with a single hapten and the reactions of the same antibody with different haptens. The concept that immunological specificity is determined by a unique recombination of pre-existing globulin molecules differing in their molecular configuration retains all the advantages of Pauling's theory for the understanding and prediction of the antibody-combining power of structurally related haptens. Both theories envisage the formation of a stable complex between antigen and protein antibody in virtue of the development of short-range inter-molecular forces resulting from the complementariness between the structure of the antigen molecule and the configuration or pattern of folding of the antibody protein or proteins. As I had remarked earlier, the theory is essentially a re-statement of Ehrlich's 'side chain' theory in the current idiom of biological thought. The process of immunization, according to both theories, consists in the accelerated production of selected constituents of the normal cell.

The actual mechanism by which the elective stimulus of an antigen effects antibody synthesis remains largely obscure. Jerne has proposed an elective transport of antibody-forming templates to the sites of globulin synthesis, but biochemical support for this is lacking. Talmage and Burnet have proposed a process of cellular selection. This appears to have been more fruitful of experimental work, particularly the study of population dynamics of mesenchymal cells in relation to the operation of immunological stimuli.

Current concepts of immunogenesis have thus revived the significance of the analogy between the processes of immunogenesis and enzyme induction. Several aspects of the functioning of enzyme induction systems have been elucidated in detail, largely due to the work of Monod's school in recent years. We may confidently expect that a clearer understanding of the role of antigen in the actual process of antibody synthesis will emerge from experimental work taking the enzyme induction system for a working model.

GROUP DISCUSSION ON TEACHING OF MICROBIOLOGY IN INDIAN UNIVERSITIES

The teaching of microbiology in Indian Universities was considered once again at the present session. The present status of the science of microbiology and its application in India was reviewed, and the training and educational facilities available in Indian Universities assessed in relation to the needs during the Third Five Year Plan for specialist personnel in the field of microbiology. Curricula and practical work for general courses in microbiology at various levels, graduate and post-graduate came in for detailed discussion. The group discussion had got off to a purposeful start with the plea Dr. N. K. Sidhanta, Vice-Chancellor of the Delhi University had made earlier at the inaugural session, for instituting systematic courses of study in microbiology as an independent science in the Indian Universities. The discussion was presided over by Dr. R. S. Vasudeva, Joint Director and Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute in the unavoidable absence of Dr. M. S. Randhawa. Twelve speakers, representing the different fields of professional study which comprise specialized courses in microbiology presented papers and several others took part in the lively discussions that ensued. We give below a summary of the important points made in the course of these discussions.

Development and future of microbiology in India

The future of microbiology in India was considered in the light of experience gained here and achievements recorded elsewhere. Microbiology has great applications in problems concerning agriculture, medicine, public health, forestry, marine and fresh water biology etc. There is a general realization that objective study and therefore teaching of microbiology has suffered a great deal in the past because chemists were primarily interested in the chemical processes in which only certain special types of microbes take part, and the pathologists in the microbes that cause human, animal and plant diseases. This contributed to the highly atypical development of microbiology unlike that of botany and zoology. This situation has been largely remedied in the West and in Russia due in no small measure to the realization in the last 20 years of the extremely important and vital role microbes play and will play in future human welfare plans. Morphology, taxonomy, ecology and physiology of microbes inhabiting the various habitats in the wider sense are being taught in most leading universities in the West and Russia today.

It is now an established fact many of the concepts of modern biochemistry have originated in work on the metabolism of micro-organisms. Microbial genetics have enriched modern genetics with new techniques and ideas. Studies on the interaction between viruses and host cells are modifying our basic notions concerning cellular individuality and integrity. In these and many other ways, microbiology is now making fundamental contributions to the concepts of biology as a whole. The study of micro-organisms has given greater insight to our understanding of the vexed problem of the origin of life. It is important therefore that during the third five year plan, every oppor-

tunity should be taken to establish separate departments in general microbiology at least in selected Universities in India, with financial support from the University Grants Commission and the State Governments concerned.

Curricula and academic standards.

The creation of separate department of general microbiology in universities will not cause any serious dislocation in the curricula of the existing universities or of the new universities coming up in the third five year plan. The department will immediately be concerned with the systematic biology, physiology, genetics, cytology and distribution of different types of micro-organisms, bacteria, microfungi, microscopic algae, protozoa, viruses etc. Sufficient attention will be paid to microbes causing disease and their control and to the teaching of important aspects of applied and industrial microbiology. The department will admit and prepare students for award of B.Sc., M.Sc. and Ph.D. degrees in microbiology.

Students admitted to the degree course in microbiology should preferably be those who have passed the Inter Science examination of the university with biology and chemistry. It is desirable to have a Standard Curricula at least for courses leading to the B.Sc degree. For instance, the university of Bombay which has pioneered the teaching of microbiology in India requires those who register for B.Sc degree to appear, all told, in 8 theory papers, 8 practical examinations and three orals. Realistic practical work is given and attempts are made to do work following established laboratory manuals. Details of the theoretical and practical courses are specified from time to time by the Academic Council on the recommendation of the Board of Studies.

It does not however seem desirable to lay down All India Standard Curricula for post graduate degrees and research. Much will depend upon facilities available at individual universities. M.Sc. and Ph.D degrees will be awarded by research alone or by combination of research and course studies, a practice much in vogue in most American Universities. This system could be adopted in India with advantage. It is probably best to offer a few advanced package courses on determinative bacteriology, microbial genetics, microbial nutrition, taxonomy of moulds, viruses, rickettsiae, serology and immunology, protozoa, blue green algae, pathogenic bacteria, elementary statistical methods, micro-organisms and soil fertility, dairy bacteriology, industrial microbiology and so on. Students will be required to take up thorough practical work based on laboratory exercises and instrumentation techniques. Research work will be followed by presentation of a dissertation.

Specialized institutions affiliated to the universities.

The feeling was unanimous that the time has come for the revision of the scope and system of teaching of bacteriology and pathology in professional institutions and colleges in the field of public health and medicine, dairy and animal husbandry, agriculture and forestry. The syllabi for pathology and bacteriology in most specialized institutions, particularly medical and veterinary, are too lopsided, and should be redrawn as early as possible, with emphasis on the independent and adequate teaching of microbiology. More than one speaker pointed out that new knowledge accumulating in the last 25 years on viruses, pathogenic fungi, prophylaxis, immunity, parasitology and epidemiology have cast a heavy burden on the department of pathology and bacteriology

which is staffed mostly by pathologists, who by reasons of their training and background are unable to cope with these growing disciplines by themselves. It was suggested that there should be at least a separate paper comprising bacteriology and virology and a paper in parasitology separate from pathology. In the case of veterinary colleges virology, parasitology, mycology and bacteriology should form separate papers. It may be necessary to have on the staff one or two extra senior teachers to take the greater teaching load. More comprehensive syllabi and more realistic laboratory manuals in these branches must be drawn up by the Board of Studies and Academic Council of the university concerned. The time is also ripe for instituting a chair in bacteriology and virology separated from pathology in medical colleges.

The group discussion concluded with a lucid summing up of the papers and discussions by the sessional president, Dr. R. S. Vasudeva. The General Assembly thereafter resolved that the teaching of general microbiology should be started immediately in Indian universities. To this end, separate departments of microbiology to prepare students for the B.Sc. and post-graduate (research) degrees in general microbiology should be opened by the universities. The Assembly also empowered the Central Council to form a special committee for formulating concrete proposals in the light of the deliberations on the teaching of microbiology in Indian universities and submit them to the University Grants Commission, Vice-Chancellors of the Indian universities and Principals of the four Indian Institutes of Technology.

List of speakers and participants

1. J. C. Ray (Calcutta), 2. B. N. Singh (Lucknow), 3. R. N. Singh (Banaras), 4. S. Sinha (Agra), 5. C. M. Singh (Mathura), 6. M. S. Das (Calcutta), 7. B. M. Gupta (Lucknow), 8. R. S. Vasudeva (Delhi), 9. W. V. B. Sundara Rao (Delhi), 10. S. C. Seal (Delhi), 11. S. S. Bhatnagar (Bombay), 12. Miss L. Rao (Ludhiana), 13. N. Chitkara (Amritsar), 14. A. Anantnarayan (Trivandrum), 15. F. Fernandes (Bombay), 16. Miss. Y. Freitas (Bombay), 17. K. S. Bhargava (Gorakhpore), 18. B. Ghosh Ray (Delhi).

SCIENTIFIC SESSIONS

Scientific papers were presented and discussed at the Congress. They were taken up in the course of six sessions namely medical bacteriology (A), veterinary and agricultural bacteriology, cholera, tuberculosis and antibiotics, viruses and virus diseases, (symposium) and medical bacteriology (B). It is noteworthy that the symposium on viruses and virus diseases brought together medical, veterinary and plant virologists on a common platform for the first time in this country. Abstracts of the papers presented are published below.

INDIAN JOURNAL OF MICROBIOLOGY

Vol. I, No. 2, 1961

ABSTRACTS OF PAPERS PRESENTED AT THE FOURTH MICROBIOLOGICAL CONGRESS

Session I

MEDICAL BACTERIOLOGY : SECTION A

Chairman—Lt. Col. Oscar Felsenfeld

1. ENTEROPATHOGENIC *ESCHERICHIA COLI* SEROTYPES IN VISAKHAPATNAM;

M. LAKSHMI NANDI, DEPARTMENT OF BACTERIOLOGY, LADY HARDINGE MEDICAL COLLEGE, NEW DELHI

Clinical material was collected from infants up to the age of 2 years (admitted into King George Hospital, Visakhapatnam) with a history of acute diarrhoea. Two hundred and twenty five cases of diarrhoea and 75 control cases were investigated.

(i) A bacteriological survey of infantile diarrhoea in general and the role of *Bact. coli* as a causal organism in particular was carried out.

(ii) The cultural characters and biochemical reactions of several variants of *Bact. coli* (typical) were studied.

(iii) A detailed study of the incidence of certain serological types of *Bact. coli* was made.

(iv) In a series of 225 cases of infantile diarrhoea *Bact. coli* (typical) was demonstrated in 140 cases (6·22%)

(v) On serotyping of the 140 strains of *Bact. coli* (typical) *Bact. coli O* group 111 was found in 49% of cases. *Bact. coli O* group 55 was found in 16% of cases. *Bact. coli O* group 26 was found in 9% of cases.

(vi) Out of the 140 strains, 37 strains (26·4%) were found non-agglutinable with any of the 3 available high-titre-sera.

2. *ESCHERICHIA COLI* SEROTYPES ISOLATED FROM SPORADIC CASES OF INFANTILE DIARRHOEA IN DELHI;

OM PRAKASH, DEPARTMENT OF BACTERIOLOGY, ALL INDIA INSTITUTE OF MEDICAL SCIENCES, NEW DELHI

One hundred and fortyfive cases of infantile diarrhoea have been examined for the *Escherichia coli* serotypes associated with them. All the *E. coli* isolated from these cases were

typed by *E. coli OB* antisera of types 0111B4, 055B5, 026 B6 and 0127 B8. *E. coli* isolated from 85 of these cases were typed with *E. coli O* antisera of types 055, 0111, 0127, 026, 0125, 0124, 0128, 0126, 0119, 086, 044, 0112, 0102, 020 and 018.

The serotypes isolated were 055 B5, 0127 B8, 0128, 0125, 018, 020 and 044.

Sorbitol fermentation and acriflavine agglutination properties have been studied for all the strains isolated.

3. A STUDY OF THE BIOCHEMICAL REACTIONS OF *SALMONELLA TYPHI* ORGANISMS ISOLATED FROM CASES OF ENTERIC FEVER; D. S. AGARWAL, DEPARTMENT OF BACTERIOLOGY, LADY HARDINGE MEDICAL COLLEGE, NEW DELHI

One hundred and ninetyeight strains of *Salmonella typhi* isolated from the cases of enteric fever were investigated as regards their biochemical reactions. Fermentation of 14 different carbohydrates and 3 organic acids, production of indole and H_2S, reduction of nitrates, growth in Simmon's citrate and Simmon's glucose, M.R., V.P., decomposition of urea, liquefaction of gelatin, growth in KCN broth and determination of amino acid decarboxylase activity were done. Biochemical sub-types of *S. typhi* based on the presence or absence of fermentation of xylose, arabinose, d-tartrate and sodium citrate have been discussed. The biochemical types of *S. typhi* phage types have been described and discussed. The biochemical reactions utilised for the purpose are dulcitol, arabinose, xylose, d-tartarate, sodium citrate, sodium mucate, H_2S and Simmon's glucose.

4. IMMUNITY LEVEL AGAINST TYPHOID AND PARATYPHOID BACTERIA IN THE INDIGENOUS VILLAGE POPULATION; H. N. DUTTA, ARMED FORCES MEDICAL COLLEGE, POONA

The communication records the results of testing random samples of 316 sera of recruits from indigenous village population against typhoid-paratyphoid bacteria. These people are from the various parts of India.

The objects of the investigation were two fold:

(i) In view of the paucity of published reports on this subject, it was considered desirable to obtain further information of the agglutinin level in normal sera

(ii) The acquisition of data for the incidence of typhoid carriers in the community by Vi-agglutination test, taking into consideration that agglutination of serum in dilution of 1·10 or over, under standard conditions, should be regarded as significant (Felix)

Three hundred and sixteen sera of recruits (all male) collected at random were examined for Vi-agglutinin, normal 'O' and 'H'-agglutinins against *S. typhi*, *S. paratyphi A* and *S. paratyphi B* by Felix's technique and Dreyer's method respectively. The percentage of people showing Vi-agglutinin in their serum and hence, the probable carrier rate, is approximately 1·9.

5. RESISTANCE OF *SALMONELLA TYPHI* TO CHLORAMPHENICOL; B. RAMANARAYANA MURTHI, K. RAJYALAKSHMI AND C. S. BHASKARAN, DEPARTMENT OF BACTERIOLOGY, GUNTUR MEDICAL COLLEGE, GUNTUR, ANDHRA PRADESH

The sheet anchor in the treatment of enteric fever has been chloramphenicol ever since this antibiotic has been introduced in the market on a large scale. Flippin and Eisenberg (1958) suggested that clinical and bacteriological relapses that occur during treatment of enteric fever with chloramphenicol are due to inaccessibility of the bacilli to the drug or the bacilli being protected in some way from the drug. There are very few reports in the literature about resistance of the bacilli to chloramphenicol, occurring in nature.

We tested 52 strains of *S. typhi* and 3 strains of *S. para typhi A* for growth in the presence of chloramphenicol in broth in varying concentrations from 0·1 μg./ml. to 500 μg./ml. It was noticed that 11 strains of *S. typhi* are resistant to chloramphenicol in concentrations from 50 μg. to 250 μg. Others were either resistant to smaller concentrations or sensitive to the lowest concentration studied. None of the strains of *S. paratyphi A* were resistant to the antibiotic in the range of 50—250 μg./ml.

It was further noticed that some of the strains show an adaptation to resist the antibiotic. These lose their resistance to the antibiotic when repeatedly subcultured in the laboratory. Others remain resistant throughout.

One of the later strains was grown in broth and filtered. The filtrate was added in a fixed amount to broth containing varying concentrations of chloramphenicol and a known sensitive strain was then inoculated. It was seen that the sensitive strain is able to grow in the presence of the drug and the filtrate of the resistant strain.

From these and other data it would seem possible that the resistant strain is producing some substance which antagonises the action of chloramphenicol or destroys it.

Session II

VETERINARY AND AGRICULTURAL BACTERIOLOGY

Chairman—Dr. C. M. Singh

6. NITROGEN FIXATION BY CELL-FREE PREPARATIONS FROM MICRO-ORGANISMS; R. N. SINGH, DEPARTMENT OF BOTANY, BANARAS HINDU UNIVERSITY, VARANASI

During the past quarter century, repeated attempts have been made to obtain cell-free nitrogen fixation with extracts from a wide variety of biological agents. Although these experiments were uniformly unsuccessful, the first report of consistent nitrogen fixation at a high level by cell-free preparations is that of Carnahan *et al.* (1960). They

demonstrated that soluble extracts of *Clostridium pasteurianum* prepared whether by autolysis of dried cells or rupture of fresh, frozen cells with a Hughes press, gave substantial levels of nitrogen fixation when supplied with pyruvate. Employing the preparative techniques of these authors, Schneider, Bradbeer, Singh, Wang, Wilson, and Burris (1960) have confirmed their significant results and in addition have obtained nitrogen fixation by extracts of *Rhodospirillum rubrum*, for the first time. Employing another method of enzyme extraction, i.e., by sonic disruption of the freshly harvested cells, Schneider, Singh, and Burris (1960) have obtained nitrogen fixation by extracts of 5 genera of blue-green algae for the first time. This paper will present the observations mainly of the blue-green algae.

7. ISOLATION OF AMMONIA-OXIDISING ORGANISMS AND THE EFFECT OF VITAMIN B_{12} ON THE PROCESS; R. B. REWARI AND W. V. B. SUNDARA RAO, INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI

Isolation of nitrifiers was started in 1958. Omeliansky's solution was passed through a column of soil continuously in the percolater and after about 20 days when there was sufficient formation of nitrite, a drop of it was plated on ammonium sulphate agar. After repeated sub-culture in Omeliansky's solution for about one and a half year one strain of nitrosomonas was obtained which did not show growth in peptone broth for more than 4 weeks. On subsequent transfer, it again got contaminated. The colonies were again plated and after repeated subculture for about a year, 3 strains were obtained which stood the test of purity. They were subcultured into ampoules of Omeliansky's solution and sealed, and luckily up to the third transfer the strains continued to remain in pure state.

During the process of isolation, partially pure strain of the organism was tested for nitrification and the effect of vitamin B_{12} on the rate of nitrification. Eight replications were kept with Vitamin B_{12} at a concentration of 1 μg/100 ml. and 8 replications with the basal solution of Omeliansky. It was observed that the rate of conversion of ammonia to nitrite was significantly increased in the presence of Vitamin B_{12}.

8. REVIVAL OF THE EFFICIENCY OF LABORATORY MAINTAINED *RHIZOBIUM* CULTURE BY THE APPLICATION OF PHOSPHATES; R. B. REWARI, INDIAN AGRICULTURAL RESEARCH NSTITUTE, NEW DELHI

Two strains of *Rhizobium* of berseem (*Triolium lexendrinum*), one about 10 year old culture (A) and the other about 1 year old culture (B) were used for this study. Two sets of pot experiments were arranged. One without phosphate and the other with phosphate. Five replications were kept in each treatment. Following were the treatments: (i) control. (ii) KNO_3 @ 60 lb N/acre control. (iii) inoculum A. (iv) inoculum B.

It was observed from the data that in the set where there was no P_2O_5 only the freshly isolated strain (B) showed greater yield and nitrogen recovery than the control. But in the case where P_2O_5 was used as a basal dressing both inoculum A and B gave significantly greater yields than the uninoculated and KNO_3 control and inoculum A and B were at part. From this, it could be inferred that the strain (A) which had lost its efficiency during continuous sub-culture under laboratory conditions regained its efficiency to the same extent as that of (B) when there was a basal dressing of superphosphate in the soil deficient in P_2O_5.

When phosphate was applied there was probably stimulation of the basic *Rhizobium* population in the soil. This was reflected in larger yield and nitrogen recovery which were equal to the nitrate control.

9. STUDIES ON THE ANTAGONISTIC ACTIVITY OF SOIL ACTINOMYCETES TO TWO PLANT PATHOGENIC BACTERIA; G. RANGASWAMI AND M. RAMALINGAM, DEPARTMENT OF AGRICULTURE, ANNAMALAI UNIVERSITY, ANNAMALAI NAGAR

Plant pathogenic microorganisms sooner or later reach the soil where millions of microorganisms are present. The pathogens may reach the soil as free cells, spores, mycelial bits, etc., or they may be added along with the host tissues and the fructifications. It is well known that in most cases the added pathogens are short lived, while in the others they live for relatively longer period.

With a view to examining the relationship of *Erwinia carotovora* (Jones) Holland, the organism causing soft-rot of vegetables and root crops and *Xanthomonas malvacearum* (E. F. Smith) Dowson, causing the angular leafspot and 'black-arm' disease of cotton with the soil micro-organisms, studies were undertaken in this laboratory. Cell suspensions of each of the bacteria as well as the infected tissues of the respective host plants were added to both sterilized and unsterilized natural soils. The changes occurring in the soil population as well as the survival period of the pathogens under different sets of condition were examined at periodical intervals.

It was observed that *E. carotovora* when added in water suspension to the sterilized soil survived for 171 days but when added to the unsterilized soil it survived only for 121 days. When the same bacterium was added along with the host tissue to the sterilized soil it survived for 79 days as against the survival period of 31 days when added to unsterilized soil.

When *Xanthomonas malvacearum* was added in water suspension to the sterilized soil, it survived for 153 days but when added to the unsterilized soil it survived only for 110 days. When the same bacterium was added along with the host tissue to the sterilized soil it survived for 62 days as against the survival period of 24 days when added to the unsterilized soil.

In the same studies it was observed that with the gradual reduction of the population of the pathogenic bacteria in the unsterilized soil there was gradual increase in the population of soil actinomycetes. Attempts were made to isolate such of those organisms which were predominant and which showed inhibitory effect in mixed populations in agar plates. Several isolations of the actinomycetes were made and 7 promising cultures

were isolated and studied in some detail. Four of them are found to be highly specific in inhibiting species of *Xanthomonas* and three of them inhibited mainly *E. carotovora*. Their capacities to produce antibiotics of the above specificity in liquid media under submerged aerated conditions have been tested and positive results obtained.

10. ULTRA-STRUCTURE OF BLUE-GREEN ALGAE; HANS RIS AND R. N. SINGH, DEPARTMENT OF ZOOLOGY, UNIVERSITY OF WISCONSIN AND DEPARTMENT OF BOTANY, BANARAS HINDU UNIVERSITY, VARANASI

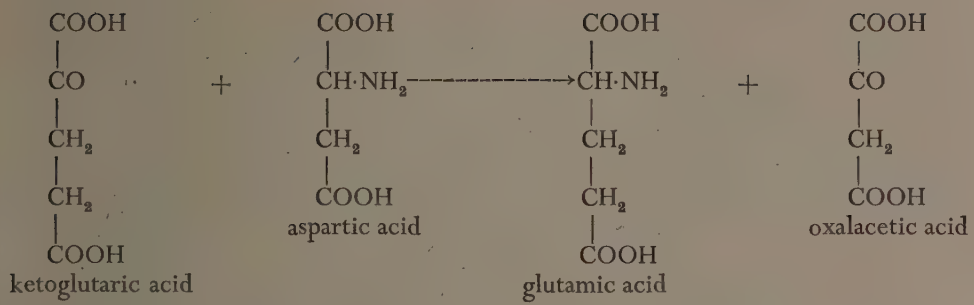
Several species of blue-green algae were studied in thin sections with the electron microscope. Our electron micrographs show that the general pattern of ultra-structure in these algae is the same as that found in bacteria and *Streptomyces*. The cells always contain photosynthetic lamellae, nucleoplasm with DNA, and small granules resembling ribosomes. The lamellae are disposed irregularly through the cell or arranged in parallel as stacks of two or more. The nucleoplasm is composed of masses of fine fibrils about 25 A thick and is either dispersed through the cell or concentrated in polymorphous reticular structures near the centre of the cell. The improved resolution makes it obvious that the terms "chromatoplasm" and "centroplasm" commonly used in the description of blue-green algae are really misleading. There are no different kinds of cytoplasm, but the cell content consists of various structural (and functional) units like the ones mentioned above, which are arranged in the cell in a number of ways characteristic for each species or for different physiological or developmental states.

11. EFFECT OF NITROGEN FIXING BLUE-GREEN ALGAE AND BACTERIA ON THE YIELD OF RICE R. B. REWARI, W. V. B. SUNDARA RAO AND G. S. VENKATARAMAN, INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI

The application of bacterial fertilizers and the nitrogen fixing blue-green algae for increased crop production is well established for some crops under certain soil conditions. During the Kharif season of 1960, the individual and combined effect of the nitrogen fixing blue-green algae, *Tolypotrix tenui* and the bacterium, *Azotobacter chrococcum* on the yield of paddy was examined in pot culture. It was found that under rice plant conditions the inoculation of the soil with algae gave significantly higher yields of paddy than the control and the series inoculated with the bacterium. The addition of phosphate and molybdenum to the algae series had no additional effect on the total yield.

12. TRANSAMINASE IN *AZOTOBACTER* SP.; S. C. CHAKRAVARTY, INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI

Transaminase activity was studied in *Azotobacter vinelandii*. The aspartic—glutamic transaminase system which catalyses the reaction



was found to be present in this organism. The activity was measured in terms of glutamic acid—N formed. Lichsterin and Cohen (1945) studied the reverse reaction.

Transamination reaction between sodium pyruvate and aspartic or glutamic acids was found to be very low and could not be measured quantitatively. The optimum *p*H value for the system is 8·0. The enzyme could be extracted from the bacterial cells.

13. OCCURRENCE OF *SALMONELLAE* IN DOMESTIC ANIMALS AND POULTRY; V. K. SHARMA AND C. M. SINGH, DEPARTMENT OF PATHOLOGY AND BACTERIOLOGY, VETERINARY COLLEGE, MATHURA, U.P.

Four enrichment *viz.*, selenite, tetrathionate, Kauffmann's modified tetrathionate and hydroquinone and three selective media *viz.*, desoxycholate-citrate agar, Salmonella-shigella agar and brilliant green agar along with polyvalent O sera (A-E), biochemical tests and *Salmonella*-genus-specific o-1 bacteriophage were used for isolation of *Salmonella* from domestic animals in carrier state.

Out of 2970 animals examined 57 were found to harbour *Salmonella* organisms. The following 17 *Salmonella* species including a new serotype named as *Salmonella mathura* with antigenic formula (9), 46 : i : enz15 were isolated during this investigation.

Sl. No.	Serotypes	Source
1.	*S. aba*	Cattle
2.	*S. anatum*	Fowls, buffaloes, pigs and rats
3.	*S. champaign*	Fowls
4.	*S. charity*	Fowls
5.	*S. chester*	Fowls
6.	*S. enteritidis* var jena	Buffaloes
7.	*S. hvittingfoss*	Fowls
8.	*S. magwa*	Cattle, buffaloes and goats
9.	*S. matopeni*	Fowls, cattle and goats
10.	*S. mathura* (newtype)	Cattle
11.	*S. newport*	Pigs
12.	*S. pomona*	Fowls, cattle and buffaloes

Sl. No.	Serotypes	Source
13.	*S. richmond*	Dead-in-shell-embryos, Fowls
14.	*S. sandigio*	Fowls and ducks
15.	*S. stanley*	Fowls and pigs
16.	*S. typhimurium*	Buffaloes
17.	*S. welteverden*	Fowls, buffaloes and goats

14. STUDIES ON COMMERCIAL LEGUME INOCULANTS; I. MICROBIOLOGICAL COMPOSITION; A. SANKARAM, DEPARTMENT OF MICROBIOLOGY, CANTERBURY AGRICULTURAL COLLEGE, CHRIST CHURCH, NEW ZEALAND

The microbiological composition of commercial peat-based legume inoculants imported into and manufactured in New Zealand was determined broadly in terms of the content of rhizobia, bacteria other than rhizobia, actinomycetes and fungi. The more dominant types under each group were isolated and identified. The effect of storage of the inoculants in a refrigerator, on the viable count of *Rhizobia* was followed at monthly intervals over a period of 7 months. The quality of the inoculants with reference to viability in storage and the nature and number of contaminants have been discussed.

15. BACTERIOLOGICAL STUDIES ON EGGS, R. A. SINGH, DEPARTMENT OF PATHOLOGY, BANARAS HINDU UNIVERSITY, VARANASI

(*i*) The present work deals with:

(*a*) the population of microorganisms present in various parts of eggs and

(*b*) the characters of the organisms present.

(*ii*) Normally fresh egg is bacteriologically sterile but contaminations occur immediately after the laying of the eggs from various external sources.

(*iii*) As expected the microorganisms are larger in number in egg shell and the egg yolk contains the least number. Both by plate and direct microscopic method egg-white contains lesser number of bacteria than the egg shell but greater than yolk.

(*iv*) Presumptive tests have indicated the presence of coliform organisms in all the market eggs indicating contamination with faecal matter; such eggs are potentially dangerous for human consumption. The presence of coliform organisms also indicates the faulty and unhygienic conditions under which the eggs were produced.

(*v*) Thirteen different types of bacteria and 2 moulds have been isolated and identified from various parts of eggs. The bacteria isolated are classified into:

(*a*) coliform organisms

(*b*) spoilage organisms

(*c*) pathogenic organisms

(*d*) non-pathogenic organisms and

(*e*) spore-forming organisms.

(*vi*) Lastly, in general, the contaminations occur from external sources after the eggs are laid. It is suggested that risk from pathogenic organisms and spoilage can be prevented by giving greater attention to the general health and hygienic conditions of poultry as also care during production and storage of eggs.

SESSION III

CHOLERA

Chairman—Dr. J. C. Ray

16. CHEMICAL STUDIES ON THE CHOLERA TOXIN AND ITS SIGNIFICANCE IN PATHOGENESIS;

M. V. PANSE, N. K. DUTTA AND H. I. JHALA, HAFFKINE INSTITUTE, BOMBAY

Evidence has been collected with the help of animal experiment that the toxin liberated by *V. cholerae* is responsible for the signs and symptoms of the disease. A study was carried out to prepare toxins by various methods and to find out their effects on infant rabbits. During the last few years a number of workers have made an attempt to fractionate cholera toxin and identify the active principle responsible for the pathological changes. They mainly studied toxicity of those fractions either on mice or on guineapigs. But none of these fractions could produce disease simulating human cholera.

In the present studies toxins which exhibited constant toxicity and choleragenic properties were fractionated to study the exact role played by each fraction. It was observed that the lipid fraction of the toxins as well as that from the *V. cholerae* was responsible for the toxic and choleragenic effects while the polysaccharides were mainly antigenic.

17. AN OUTBREAK OF EL TOR DIARRHOEA IN THAILAND; OSCAR FELSENFELD AND S. MUKERJEE,

SEATO CHOLERA MEDICAL RESEARCH LABORATORY, BANGKOK, THAILAND AND INDIAN INSTITUTE FOR BIOCHEMISTRY & EXPERIMENTAL MEDICINE, CALCUTTA

During the closing months of 1960, an outbreak of diarrhoea with vomitting occurred in Ubol, Thailand. The disease was relatively mild. There were no fatalities. El Tor

vibrios of the Ogawa subtype, belonging to Heiberg group I, were isolated from faeces, vomitus, water and food. While El Tor vibrios were found in sporadic instances in Thailand also before this outbreak, this is the first known instance when such organisms were cultured from a large group of diarrhoeic patients in that country.

18. EVALUATION OF TESTS FOR DIFFERENTIATING *VIBRIO CHOLERAE* AND EL TOR VIBRIOS

S. MUKERJEE AND U. K. GUHA ROY, DIVISION OF MICROBIOLOGY, INDIAN INSTITUTE FOR BIOCHEMISTRY & EXPERIMENTAL MEDICINE, CALCUTTA

El Tor vibrios co-exist with *V. cholerae* in natural water sources and patients' stools in cholera-endemic areas. It is therefore essential that the two species of vibrios should be differentiated in routine diagnostic tests. El Tor vibrios are identical with *V. cholerae* in their serological and biochemical reactions. The haemolytic properties of El Tor strains are usually utilized to differentiate them from the non-haemolytic cholera vibrios. But in some freshly isolated strains of El Tor vibrios the haemolytic properties may be absent and then this test fails to reveal the true identity of the strains. A number of tests, like sode-serum-agglutinatin, soda-sublimate precipitation and heat or chloroform inactivation of agglutinability have been developed to solve the problem. Recently in our laboratory it has been found that it is possible to differentiate the two species of agglutinable vibrios by their pattern of susceptibility to a group IV cholera bacteriophage.

19. THE PROTECTIVE ANTIGENS IN CHOLERA (INABA) VACCINE ; SHARDA H. ASWANI AND S. S. RAO, HAFFKINE INSTITUTE, BOMBAY

The Haffkine Institute's cholera vaccine prepared from Inaba strain was first of all analysed by gel diffusion and immunoelectrophoresis for its antigenic composition. The vaccine was found to contain 4 heat stable antigens and 7 heat labile antigens. The protective antigen as determined by the mouse protection test was found to be heat stable.

Isolation of the protective antigen was carried out from boiled vaccine by first precipitating all the antigens by saturation with ammonium sulphate. The precipitate was dried and extracted with different solvents such as, phenol, glycols, urea, glycerine, acids, alkalies, etc. The ethylene glycol extracted the most active protective antigen and was found to contain only two components as tested by gel diffusion test.

20. STUDIES ON THE ANTIGENIC RELATIONSHIP OF NON-AGGLUTINATING AND AGGLUTINATING VIBRIOS BY GEL DIFFUSION ; S. N. GHOSH AND S. MUKERJEE, DIVISION OF MICROBIOLOGY, INDIAN INSTITUTE FOR BIOCHEMISTRY & EXPERIMENTAL MEDICINE, CALCUTTA

An analysis of the antigenic structures of 16 NAG vibrios and 4 *V. cholerae* strains by gel diffusion and intragel absorption tests revealed the following:

(1) Non-agglutinating and agglutinating vibrios possess separate antigenic fractions for their species only. Each of the two sero-types of *V. cholerae* has its own specific antigenic fraction. The specific antigenic fraction of NAG vibrios differed in different strains

(2) More than one common antigenic fractions were shared by the non-agglutinating and agglutinating vibrios, of which only one was heat-stable and was different from species-specific somatic antigen

(3) There are usually two or more heat-labile fractions common in NAG vibrios and *V. Cholerae.*

21. NUTRITIONAL REQUIREMENTS OF CHOLERA INABA AND OGAWA STRAINS USED IN THE PRODUCTION OF VACCINES; M. G. NERURKAR AND S, S. RAO, HAFFKINE INSTITUTE BOMBAY

The nutritional requirements of cholera strains, Inaba 569BK and Ogawa 5321, which are used in the production of cholera vaccine at the Haffkine Institute were investigated. The requirements of amino acids, purines and vitamins were investigated using basal minimal medium consisting of ammonium sulphate (0·1%) glucose (0·1%), $MgSO_4 . 7H_2O$ (0·02%), Nacl (0·5%) and K_2HPO_4 (0·1%) and minimal inoculum. The two strains did not require any of the vitamins or purines for stimulation of growth. These strains could grow on the minimal medium supplemented by any of the amino acids, leucine, glutamic acid, arginine, lysine, or histidine. Growth was delayed in the case of amino acids, isoleucine, tryptophan and serine.

22. VARIATIONS IN THE PREVALENT PHAGE TYPES OF VIBRIOS IN CALCUTTA EPIDEMICS S. MUKERJEE, U. K. GUHA ROY, B. C. RUDRA AND A. R. DUTTA, DIVISION OF MICROBIOLOGY, INDIAN INSTITUTE FOR BIOCHEMISTRY & EXPERIMENTAL MEDICINE, CALCUTTA AND INFECTIOUS DISEASES HOSPITAL, CALCUTA

Cholera is endemic in Calcutta. Starting at a number of foci of infection the disease spreads to various parts of the city giving rise to widespread epidemics with periodic fluctuations in the incidence of cases. In these foci the different phage and sero-types of vibrios survive. The variations in the incidence of different phage and serotypes in different epidemics depend on conditions suitable for the spread of infection from these black-spots.

During the course of our study it has been observed that in 1955 Type 1 Inaba vibrios constituted about 90% of the total strains isolated; but during the period between 1956 and 1st half of 1960 phage type 1 Ogawa vibrios formed the predominating type and amounted to about 91% of the total isolated strains. After this period isolation of phage type 3 Inaba vibrios from cases became more frequent and it progressively outnumbered the Ogawa type 1 vibrios untill February 1961, when 84% of the isolated strains were found to belong to the former type.

In earlier part of this phage, the origin of the epidemic due to phage type 3 Inaba vibrios could be traced to a group of cases occurring within a short period in the Corporation ward No. 2. But as the epidemic progressed, infections due to this type of vibrio spreaded in other parts of the city and got mixed up with other vibrio types.

The sudden change-over of the predominating type from Ogawa type 1 to Inaba type 3 is remarkable. The simultaneous two-way variations in serological and phage types of *V. cholerae* lends support to the view that periodic changes in serotypes as also in phage types of the prevalent strain in a geographically localized area take place as a result of selective multiplication and spread of one type of the strains rather than through mutational changes by actions of immune sera in hosts of bacteriophages in environmental sources.

23. MODIFICATION OF BANDI'S TEST FOR THE QUICK DIAGNOSIS OF CHOLERA ; B. M. PAUL

CENTRAL LABORATORY, CORPORATION OF CALCUTTA, CALCUTTA

Bandi's test:

To a tube with a bulb at the bottom containing 15 ml. of pepton water, *p*H 8·2 was added such an amount of high titre cholera "O" serum that the final dilution of the serum in the medium was brought to about 50% of its original titre. Suspected cholera stool was added to it and examination made hourly during 3-7 hr. incubation. In positive cases granules due to agglutination of vibrios would appear in the body of the fluid and also on the sides of the tubes within 7 hr.

But it was observed that the test was useful when *V. cholerae* preponderated in the stool whereas preponderance of coliform organisms considerably affected the results. Presence of a large number of coliform organisms in the stools overgrew vibrios, made the media sufficiently turbid and musked the clumping of vibrios.

Modification:

Later on, working on various methods to overcome the difficulties of reading the granules in turbid tubes, it was observed that on further incubation of the tubes, the granules which were fine and floating in the media, became bigger and heavier and ultimately settled at the bottom of the tubes when the tubes were incubated in perpendicular position and that these granules deposited on the lower surface of the slant tubes when these tubes were incubated on slant position within 10-12 hr.

Two hundred and twenty four samples of stool of suspected cases of cholera were examined in the Central Laboratory of the Corporation of Calcutta during the post-epidemic period of 1954 when the incidence of cholera was low and the cases were mild, by this method and also by direct plating on bile salt agar media. Of these, 18 samples were positive by both the tests. Two more samples were positive by the modified method and negative by direct plating, confirmed subsequently by plating the enriched fluid media. The rests were negative to both the tests.

Session IV

TUBERCULOSIS AND ANTIBIOTICS

Chairman—Dr. R. Viswanathan

24. TESTING OF ANTITUBERCULAR DRUGS *IN VITRO* ; ANIMA E. BENDEALLY AND R. A. BELLARE, HAFFKINE INSTITUTE, BOMBAY

Antitubercular activity of 100 new compounds such as esters of p-amino-salicylic acid, thiazolyl-thioureas, pyrimidyl-thioureas, acyl and acyl-alkylthio-semicarbazides etc. was tested *in vitro* against the human virulent strain of *Mycobacterium tuberculosis* (H37Rv). The tests were carried by our using Youman's medium (modified from Proskauer and Beck medium) containing 10% horse serum.

25. GUINEAPIG-VIRULENCE OF INDIAN TUBERCLE BACILLI ; BALBIR SINGH, MAULANA AZAD MEDICAL COLLEGE, NEW DELHI

One hundred and twelve Indian strains of *M. tuberculosis* were isolated from patients suffering from pulmonary tuberculosis. Fourteen strains of saprophytic acid-fast bacilli were tested for guineapig-virulence. The results were compared with the observations made with 9 reference strains obtained from U.S.A. or France.

Only 27% of the freshly isolated pre-treatment strains of *M. tuberculosis* sensitive to streptomycin, P.A.S. and isoniazid were as virulent to guineapig as were the reference strains. This character was stable on repeated subculture or on passage through guineapig 2 or 3 times.

Nine strains tested with the Indian guineapigs were also tested in guineapigs in Germany. The results of strains in German guineapigs were similar to those already observed with the Indian animals.

26. LEISHMANICIDAL ACTIVITY OF NYSTATIN, A POLYENE ANTIFUNGAL ANTIBIOTIC. PART I. : THE PROBABLE MECHANISM OF ACTION OF NYSTATIN ON *LEISHMANIA DONOVANI* B. K. GHOSH AND A. N. CHATTERJEE, INDIAN INSTITUTE FOR BIOCHEMISTRY AND EXPERIMENTAL MEDICINE, CALCUTTA

Nystatin, a polyene antifungal antibiotic was found to have a high leishmanicidal activity on resting cell suspension of *L. donovani*. Both aerobic and anaerobic metabolism of this organism were inhibited and the inhibition pattern was similar in the presence or absence of different exogenous substrates. This inhibition of cell metabolism was accompanied by the lysis of the cells and release of the intracellular components in the incubation medium. The optimum experimental conditions for inhibition of metabolism

as determined by manometric studies were also the same under which maximum release of the intracellular components and corresponding lysis of the cells occurred. It is concluded that the primary action of nystatin is on the cell wall or membrane of *L. donovani* causing an osmotic imbalance leading to the lysis of the cells.

27. LEISHMANICIDAL ACTIVITY OF NYSTATIN, A POLYENE ANTIFUNGAL ANTIBIOTIC. PART II : ISOLATION OF THE BOUND NYSTATIN FROM THE CELLS AND ITS CLINICAL APPLICATION ; A. N. CHATTERJEE, AND B. K. GHOSH, INDIAN INSTITUTE FOR BIOCHEMISTRY AND EXPERIMENTAL MEDICINE, CALCUTTA

Resting cell suspensions of *Leishmania donovani* were incubated with nystatin for 1 hr. in normal saline, and then the lysed cell mass was washed repeatedly with saline to remove any adhering nystatin. The cells were then fractionated by centrifugation by the density gradient technique. Nystatin was found to be concentrated in a particular layer which from its chemical and microscopic analysis appeared to be the membrane layer. A sterol which we have detected in this organism is also highly concentrated in this layer. As it is known that sterols can combine with this group of antibiotics, it is possible that the receptor site of nystatin on the cell is a sterol component on the cell wall or membrane.

A successful clinical trial of a case of dermal leishmaniasis was given by oral therapy of 4 tablets per day of Mysteclin V (Squibb product containing tetracycline and nystatin) to a patient. Within two weeks of commencement of therapy, living leishmania organisms could no longer be detected in his system and after about a month the nodules also subsided. A second clinical case is now under investigation.

28. ANTIBIOTIC—RESISTANT STAPHYLOCOCCI ; S. C. PAUL AND B. GHOSH RAY, DEPARTMENT OF BACTERIOLOGY, ALL INDIA INSTITUTE OF MEDICAL SCIENCES, NEW DELHI

A study has been undertaken to determine the emergence of antibiotic-resistant strains of *Staphylococcus aureus* consequent to the use of antibiotics in hospital practice. The sensitivity pattern of 250 strains of *S. aureus* isolated from various sources were determined using penicillin, streptomycin, chloramphenicol, aureomycin, terramycin, achromycin, erythromycin and bacitracin. The large majority of strains were resistant to penicillin, whereas all the strains were susceptible to erythromycin and bacitracin. Resistance to streptomycin and tetracyclines was observed in a moderate number of strains. Only a few strains were resistant to chloramphenicol. Complete cross resistance was noticed with the tetracycline group of drugs. The relative incidence of resistant strains was correlated to the frequency with which these antibiotics were used in hospital practice. About hundred strains were also tested, against the new penicillin product "Celbenin" (BRL 1241) and the importance of the findings was discussed.

29. RAPID ANTIBIOTIC SENSITIVITY TESTS—A SIMPLE MODIFICATION ; J. M. MOSES, B. N. JOSHI, V. H. KALGI AND R. K. GADGIL, DEPARTMENT OF PATHOLOGY AND BACTERIOLOGY, GRANT MEDICAL COLLEGE, BOMBAY

A simple modification in the dye methods of rapid antibiotic sensitivity testing using a combination of methylene blue and azure is described.

The results are compared with the standard tube dilution method.

A comparative study of the said method and other rapid methods is presented.

Session V

SECTION A. ANIMAL VIRUS

Chairman—Dr. C. G. Pandit

30. TRENDS IN RESEARCH ON VIRUSES AND VIRUS DISEASES IN INDIA

Inaugurating the symposium Dr. C. G. Pandit remarked that regular prophylaxis against small pox, rabies and Ranikhet disease virus of fowl has been practised in this country for a long time. It is only in the beginning and in the intervening years, following World War II, that studies on some of the known virus diseases and identification of new ones, affecting humans, vegetable crops and animals have been made. Studies pertaining to incidence, serodiagnosis, healthy carrier state, laboratory cultivation, host range and insect transmission of viruses have received more attention. Study of virus infection at cell level, the centre of virologists' present interest, has been so far more or less neglected. Infectivity studies of viral nucleic acid, isolation of tissue cell lines (animal, human or plant) and the use of bacteriophage as model for conducting metabolic studies pertaining to growth, reproduction, and inhibition of viruses have received little attention. This has been primarily due to the lack of trained personnel. The Indian Council of Medical Research has immediate plans to introduce a large number of National Junior and Senior Fellowships in virology, tenable at different institutions in India, in order to encourage research in this branch of science. Plans to set up more virus diagnostic laboratories are also under way. Dr. Pandit congratulated the Association of Microbiologists of India and the organisers of the symposium for making it possible for plant and animal virologists in India to come together for the first time and to present the type of work that they are doing.

31. THE PROGRESS IN RESEARCH OF ARTHROPOD-BORNE VIRUS IN INDIA ; S. ANDERSON, VIRUS RESEARCH CENTRE, POONA

The Virus Research Centre was established to investigate virus infections of man and animals with particular emphasis on the arthropod-borne viruses. The virus problem has been one of determining the prevalence and importance of the many previously known

arbor virus infections and of discovering new ones. The second problem has been the training of Indian personnel in this branch of scientific research.

The programme has been divided into two closely interdependent activities, the field and the laboratory. Since the problems to be investigated are concerned mainly with the epidemiology of the virus, the field programme has determined the direction the laboratory investigations will take.

Field studies in the arbor virus field demand the participation of several specialists such as entomologists, epidemiologists, ecologists, etc. As a result of these studies a number of different arthropod-borne virus infections in India have been identified. Among these are infections due to the Japanese B encephalitis virus, dengue, West Nile, Sindbis, African horsesickness, and several as yet unidentified viruses which may be identical with other known viruses or related to them or may be viruses as yet unknown to science. At the present time the Virus Research Centre operates two field stations, one for the study of KFD and the other for JBE.

The laboratory studies are undertaken with the view of backing up, so to speak, the field effort and also to provide information concerning the field problems which can only be obtained in the laboratory. The laboratory consists at present of a serology, virology, entomology and tissue culture section.

32. ENTEROVIRUSES AMONG HEALTHY INDIVIDUALS OF BOMBAY ; K. M. MEHERHOMJI, GRANT MEDICAL COLLEGE, BOMBAY

Stool samples were collected from apparently healthy individuals belonging to different age groups and to both sexes. Collection was done in winter as well as monsoon months. Of 232 stool samples collected and inoculated in monkey kidney tissue cultures, 32 (13·8%) cytopathogenic (CP) agents were isolated. No additional viral agent was found by inoculation in infant mice.

Serologically the enteroviruses were indentified as follows:

Nine as polioviruses (1 type 1, 2 type 2, and 6 type 3), seven as Coxsackie viruses (5 type A9, and 2 type B4) and ten as ECHO viruses (4 type 1, 3 type 3 and 3 type 11). Six cytopathogenic agents have remained unidentified.

Distinguishable plaques were obtained for the three virus groups.

Of the nine polioviruses inoculated in monkeys (rhesus) by the intracerebral route, seven were found to be paralytogenic. Two appear to be non-paralytogenic.

Of the ten strains inoculated in embryonated eggs by the allantoic route, one was successfully adapted.

33. STUDIES ON RANIKHET DISEASE VIRUS OF FOWLS ; O. P. BABBAR, CENTRAL DRUG RESEARGH INSTITUTE, LUCKNOW

Recently Tamm and others at the Rockefeller Institute, have demonstrated that anti-polio and anti-influenzal activity of certain benzimidazole glycosides is somewhat related to its chemical structure and their antiviral action does not extend to DNA viruses e.g. vaccinia virus. Antiviral action could be increased manyfold by appro-

priate alteration in the structure of the benzimidazole glycoside without inflicting any corresponding tissue or cell damage. A number of derivatives of ribonucleotides (adenylic acid), pyrimidine homologues have been synthesised at the Central Drug Research Institute, Lucknow. Their effect on the virulence of Ranikhet disease virus for chick embryo, fowls and tissue cells have been studied. It has been found by us that adenylic acid (adenosine-3-phosphate) under certain conditions, reduced the cytopathic activity of the virus without affecting its ability to produce the haemagglutinins. Adenylic acid gives full protection to fowl when administered 1 hr. before or after inoculation of a lethal dose of the virus. Tests done in infected chick embryo show that the degree of anticytopathic activity of adenylic acid depended upon its concentration in relation to the virus. Virus released from the infected cultures of chick chorioallantoic membrane, treated with adenylic acid, does not cause mortality of the chick embryos. Repeated passage, however, restores the virulence of the virus for the chick embryo. There is evidence that C_{14}-adenylic acid is incorporated into the viral component (RNA fraction).

34. SEROLOGICAL EVIDENCE OF SENDAI VIRUS INFECTION IN KERALA ; R. ANANTHANARAYANAN AND C. K. JAYARAM PANIKAR, DEPARTMENT OF BACTERIOLOGY, MEDICAL COLLEGE, TRIVANDRUM

Serological evidence of infections by Sendai virus in Kerala has been studied. It is found that antibodies to Sendai virus by HAI test are demonstrable in the sera of the population of Kerala. The titre and extent of antibodies are considerably lower than in the populations of other countries. It is felt that mere demonstration of antibody, unsubstantiated by virus isolation, cannot be taken as absolute proof of evidence of infection by Sendai virus.

35. SURVEY OF MUMPS AND SENDAI VIRUS ANTIBODIES IN DELHI ; C. K. JAYARAM PANIKAR, DEPARTMENT OF BACTERIOLOGY, ALL INDIA INSTITUTE OF MEDICAL SCIENCES, NEW DELHI

Haemagglutination-inhibiting and complement-fixing antibodies to Mumps and Sendai viruses were estimated in sera received for routine serological examinations. While in general, the results confirmed the close association of the antibodies with both the viruses, in several cases antibodies to either virus occurred alone. Sera from immunised animals failed to reveal any antigenic relationship between the two viruses. Paired sera from a Mumps patient did not show any rise of Sendai virus antibodies.

The results indicate that antibodies to Sendai virus in human sera are not always the consequence of mumps infection and that infections due to Sendai virus or some other antigenically related virus are prevalent in the local population.

36. GRANULAR VULVOVAGINITIS IN RUMINANTS ; G. BISWAL AND S. N. PANDA, ANIMAL HUSBANDRY AND VETERINARY SERVICES, BHUBANESWAR, ORISSA

The present paper puts on record the incidence of granular vulvovaginitis in cattle, buffaloes, goats and sheep for the first time in Orissa. Except probably in buffaloes,

this also is the first authentic record of this disease in India. In some of the farms, Goshalas and clinics, examination of the vulva and vagina of different species of animals revealed a very high incidence of vulvovaginitis to the tune of 82·9 to 100·0% in cattle, 46·4 to 66·6% in buffaloes, 22·7 to 33·3% in goats and 100·0% in sheep. The disease occurred in animals of all age groups, except in very young animals and in majority of cases examined, the disease was found to occur in a very severe form. A purulent discharge, probably associated with pyaemic infection, was seen in several animals suffering from vulvovaginitis.

The gross pathological lesions seen in the vulva and vagina of affected animals have been described and their significance in the diagnosis of the disease has been discussed.

The diagnosis of its virus origin was based on negative bacteriological findings.

SECTION B. PLANT VIRUS

37. TRENDS IN RESEARCH ON PLANT VIRUSES AND VIRUS DISEASES IN INDIA ; R. S. VASUDEVA, INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI

Viruses have come to occupy a very important place in the human economy due to the diseases they cause in man, domesticated and wild animals, insects, plants and even bacteria. They have been a scourge of mankind since before the dawn of recorded history. Small pox, for example, existed in China in 1700 B.C. Measles, mumps, influenza and scarlet fever are some of the other virus diseases that affect human beings. Next in importance are the virus diseases of plants which indirectly affect the common man by robbing him of his due share of food.

The virus diseases are responsible for serious losses to our agricultural crops. They are all the more important in the plantation crops as also those which are propagated vegetatively. Ever since the discovery of tobacco mosaic virus in 1892, over 300 different viruses attacking plants have been described. Most of the economic crops are affected with one or the other virus. Although accurate figures of losses due to these are not available it is now well known that the damage they cause probably equals that due to all other disease-causing agents. Due to the serious nature of these diseases, plant virus diseases have received considerable attention in other countries and the science of Virology has made considerable progress. The most outstanding advances made are the isolation and crystallization of several viruses, studies of their chemical composition, biochemical studies and the use of serological methods in the identification and differentiation of viruses and virus strains.

The science of Virology is comparatively of recent origin in India. Although the spike disease of sandal was reported to be graft transmissible by Coleman as early as 1917 systematic research on plant viruses in India started only about a quarter of a century ago. During this period several diseases of economic crop plants, fruits and vegetables as well as plantation crops have been investigated in order to evolve suitable methods of their control. Among the earliest described diseases is the tobacco leaf curl, a virus that is transmitted in nature by the white fly (*Bemisia tabaci* Gen.) and has a very wide host range which includes tomato, papaya, chilli, Sann-hemp and a large number of

weeds and ornamental plants. The potato crop has been reported to be affected by several viruses in India such as potato virus X, Y, A, D, and leaf roll which are responsible for heavy losses in yield and degeneration of seed stocks. Sugarcane mosaic 'little leaf' of brinjal and yellow vein mosaic of *Bhindi* are some other diseases that were described in early days.

A comparatively rapid progress has been made during the last fifteen years and a large number of virus diseases such as mosaic of Bottlegourd, Sann-hemp, papaya, cardamon, lettuce, cowpea and soyabean, 'grassy shoot' of sugarcane, mosaic and bunchy top of banana, 'Foorkey' and 'Chirke' diseases of large cardamon, 'small leaf' of cotton, sesamum phyllody, coconut wilt and citrus decline have been described and their vectors reported. The study of virus diseases of stone fruits have also received attention and 'line pattern' of plum, plum mosaic, marble mosaic of cherry, mosaic of *Rubus ellipticus*, peach mosaic, variegated mosaic of apple and 'line pattern' of almond are some of the diseases recorded so far.

Although most of the earlier work on plant viruses in India relates to the study of the symptomatology and host range, determination of vectors and identification of the viruses, during the recent years attempts are being made to tackle the problems of fundamental nature. Virus-vector relationship studies have been conducted with respect to a number of virus diseases. Studies on the purification of plant viruses and their biophysical and biochemical properties have been initiated. The shape and size of the virus particles is being studied. The importance of serological studies on plant viruses which had received little attention in the past has now been recognised. It has now developed into an important branch of virus study and is a valuable tool in differentiation and identification of viruses and virus strains.

As the knowledge about plant viruses is rapidly increasing, new techniques or procedures are being devised to replace the existing methods of control. While the seed certification and the use of virus-free seed potatoes is receiving wider application and resistant or tolerant varieties have been determined for yellow vein mosaic of *Bhindi*, chilli mosaic, 'small leaf' disease of cotton and papaya mosaic, the possibilities of use of heat and chemotherapeutic treatments in the control of plant virus diseases are being explored. In this respect studies on the inhibition of viruses by various chemicals, antibiotics and other agents have been initiated. The recent introduction of systemic insecticides has opened new opportunities for the direct control of insect vectors and a number of systemic insecticides such as Ekatox and Folidol have been included in the trials for control of tomato leaf curl and other virus diseases at the Indian Agricultural Research Institute.

The control of plant viruses by inoculating plants with mild or attenuated strains of viruses with a view to protecting against infection with severe ones is another measure which holds promise in the future. Although in this method of control there are chances of contaminating the crop besides having the danger of synergistic effects, of chance infection with other viruses, the method needs to be given a fair trial as it would perhaps be a lesser evil.

Another useful line of control which has been mostly neglected till today is the biological control of plant viruses. It is well known that several fungi and viruses parasitise the insects and this fact could be exploited in the interests of the agriculturist. It would,

therefore, be of interest to study the fungal and virus diseases of insects particularly the vectors with a view to explore the possibilities of biological control.

The research on plant viruses in India has so far been chiefly confined to the Indian Agricultural Research Institute and a few other central Agricultural Institutions in the country. It is high time that these studies are taken up at University level and by the States. A beginning has already been made in this direction and it is hoped that adequate facilities will be provided in the State Institutes as also Universities. What is required is a team work by plant pathologists, biochemists, physicists, plant physiologists and geneticists to solve some of our more intricate problems e.g., root diseases of coconuts which are widespread in coconut-growing countries of the world and have almost shattered their economy.

38. MULTIPLICATION OF COLIPHAGE (CVX-5) IN *ESCHERICHIA COLI* ; B. M. GUPTA, CENTRAL DRUG RESEARCH INSTITUTE, LUCKNOW

Coliphage (CVX-5) was isolated from bacteria-free filtrate of a faeces of a patient of colitis. It produced big and small plaques when plated on *Escherichia coli* (Kasauli strain) but only small plaques on *Shigella shigae* and *Eberthella typhi*. *Vibrio cholerae* and *Staphylococcus aureus* were not lysed. The phage was purified by single plaque technique and differential centrifugation.

The main features of plaque formation by CVX-5 on *E. coli*—the time of first appearance of plaque, the rate of plaque formation, period of peak production and 50% yield time—were studied. The lag period was estimated between 135 and 165 min., the period of peak production between 195 and 225 min. and 50% yield between 202 and 316 min. The rate of plaque formation works out to 21-35% per unit increase in log time. Most of the phage-resistant strains isolated were purine-requiring. Adenylic acid (adenosine-3-phosphate) increased the plaque count and plaque size considerably, when it was incorporated into the host agar plate prior to incubation.

39. A VEIN-BANDING MOSAIC DISEASE OF CHILLIES (*CAPSICUM FRUTESCENS* L.) ; K. S. BHARGAVA AND R. D. JOSHI, DEPARTMENT OF BOTANY, GORAKHPUR UNIVERSITY, GORAKHPUR, U.P.

A vein-banding mosaic disease of chillies (*Capsicum frutescens* L.) found prevalent in fields near Bhowali has been studied. The causal virus is mechanically transmitted to different commercial varieties of chillies, and also to other solanaceous plants. *Myzus persicae* Sulz. and *Aphis gossypii* Glov. can transmit the disease after short infection-feeding. It is not transmitted through seeds of chillies. It resembles potato virus Y in physical properties and host-range, but differs from it in its reaction towards tobacco in which it induces necrosis of veins, petiole and stem. Serologically it is related to potato virus Y, but both do not cross-immunize in tobacco plant. It is, therefore, regarded as a variant strain of potato virus Y similar to tobacco veinal necrosis virus.

40. INSECT TRANSMISSION OF CHILLI MOSAIC DISEASE; T. K. NARIANI AND K. S. M. SASTRY, INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI

It has been found that a single viruliferous aphid (*Aphis gossypii* Glove) is capable of transmitting the virus to healthy plants. The aphids could transmit the virus after feeding on infected plants for 30 sec. but the maximum infection was obtained when feeding period was 5 min. The infectivity of the viruliferous aphids decreased with longer feeding intervals. Previous fasting of the aphids increased their efficiency in transmitting the virus in the case of short acquisition feeding.

41. POTASSIUM NUTRITION OF TURKISH TOBACCO (*NICOTIANA TABACUM* L.) PLANTS IN RELATION TO MULTIPLICATION OF TOBACCO MOSAIC VIRUS; G. S. VERMA AND J. P. VARMA, DEPARTMENT OF BOTANY, LUCKNOW UNIVERSITY, LUCKNOW

Turkish tobacco (*N. tabacum* L.) was used as host plant. Arnon and Hoagland's nutrient solution with 0, 39, 225 and 390 ppm concentrations of potassium was utilised as nutrient solution. In each set of experiments, when potassium deficiency symptoms of intervienal chlorosis and stunted growth of plants was evidenced, half the number of plants were inoculated with tobacco mosaic virus which in course of a week exhibited systemic disease symptoms and an equal number of plants were inoculated with sterile distilled water (controls). In both healthy and diseased plants fresh weight increased regularly with increasing concentrations of potassium, being heighest at 390 ppm while height was relatively less affected. Leaf-stem ratio remained irregular in relation to differential potassium nutrition, although it increased in diseased plants. Virus concentration in leaf and stem juice when estimated by counting the local lesions produced on half leaves of test plant (*Nicotiana glutinosa* L.) and weighing the dried virus protein did not show the same relation as exhibited by fresh weight, height or leaf stem ratio. Supplements of 225 and 390 ppm to potassium deficient plants enhanced the fresh weight but failed to increase the virus concentration. The results indicated that variations in potassium supply had marked effects on host growth but not on virus multiplication except when very high amounts of this nutrient were supplied. Total nitrogen and total soluble protein contents were consistently greater in leaf juice of diseased than of their healthy counterparts following the pattern of virus concentration.

42. NITROGEN NUTRITION OF TURKISH TOBACCO (*NICOTIANA TABACUM* L.) IN RELATION TO MULTIPLICATION OF TOBACCO MOSAIC VIRUS; J. P. VARMA, DEPARTMENT OF BOTANY, LUCKNOW UNIVERSITY, LUCKNOW

Height of stem and fresh weight per plant of Turkish tobacco (*Nicotiana tabacum* L.) grown in purified sand were studied by supplying zero, low, medium and high amounts of nitrogen in nutrient solution. Typical deficiency symptoms remained prominent in plants receiving zero and low amounts of the nutrient. Host plant response to various concentrations of nitrogen in nutrition was also usually similar even after tobacco

mosaic virus infection. Virus concentration, estimated by local lesion count method in expressed saps from leaf tissue samples, regularly increased with increasing amounts of nitrogen in nutrient solution supplied to the host plant. The direct relationship of tobacco mosaic virus with amounts of nitrogen was so consistent that even on withholding nitrogen supply to plants previously grown at higher levels, or on supplementing it to deficient ones, it existed almost parallel to available nitrogen irrespective of the height, fresh weight and leaf-stem ratio of the host plant.

43. MAGNESIUM NUTRITION OF TOBACCO AND PETUNIA PLANTS IN RELATION TO MULTIPLICATION OF TOBACCO MOSAIC VIRUS; J. P. VARMA, DEPARTMENT OF BOTANY, LUCKNOW UNIVERSITY, LUCKNOW

Turkish tobacco (*Nicotiana tabacum* L.), *N. glutinosa* L. and Petunia (*Petunia* sp. L.) used as host plants, were grown in sand cultures watered with Arnon & Hogland's nutrient solutions containing 3 different levels *viz.* 0, 48, and 480 ppm of magnesium. When magnesium deficiency symptoms of uniform yellowing appeared on very small leaves and stunted growth of plants became evident, half the number of host plants were inoculated with tobacco mosaic virus, rest were left uninoculated (controls). It was found that magnesium affected the height and fresh weights of both inoculated and uninoculated host plants; height and weight were maximum at 48 ppm, leaf-stem ratio being irregular.

With the increase of magnesium nutrition of host plant the virus concentration, measured by spectrophotometric and local lesion count method, showed a simultaneous increase. The height, fresh weight and leaf-stem ratio in the plant did not correspond to or show any relationship with virus multiplication. This relation of magnesium with tobacco mosaic virus concentration was consistently exhibited when amounts of this nutrient in nutrition were altered variously during the course of experiments.

44. YELLOW-NET VIRUS DISEASE OF TOBACCO PLANT; K. L. DHINGRA AND T. K. NARIANI, INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI

A disease of tobacco plant, characterised by severe veinal chlorosis, yellow network of veins and veinlets, was found to be caused by a virus. The virus could be transmitted from diseased to healthy plants by grafting and also by white fly (*Bemisia tabaci* Gen). It also attacked *Beta vulgaris* and aster plant. Cross protection tests showed that the yellow-net virus is related to tobacco leaf curl virus. It appears to be a new virus.

45. ANTIGENICITY OF PURIFIED BOTTLE GOURD MOSAIC VIRUS; G. P. ANAND AND M. D. MISHRA, INDIAN AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI

Purified preparation of the virus was intravenously injected in albino rabbits at weekly intervals. The antiserum obtained from the immunised rabbit was found to give

a clear flocculent precipitate with the sap of diseased plants but not with the sap of healthy plants. The optimum precipitation occurred at 1 : 2 dilution of antiserum and 1 : 4 dilution of the virus.

SECTION C. BACTERIAL VIRUS

46. PHAGE TYPING OF *ESCHERICHIA COLI* OF ANIMAL AND HUMAN ORIGIN

S. S. KASATIYA AND C. M. SINGH, DEPARTMENT OF PATHOLOGY, VETERINARY COLLEGE, MATHURA, U.P.

Studies were conducted on the phage pattern of 138 strains of *Escherichia coli* from animals and 62 strains from human sources including strains associated with infantile diarrhoea from Christian Medical College & Hospital, at Vellore and strains isolated from cases of appendicites from Creighten Freeman Christian Hospital, Vrindaban. Forty three *E. coli* phages were isolated locally from faecal samples of man and animals and were compared with 21 foreign phages including 1 human phage obtained from Central Drug Research Institute, Lucknow, 13 animal phages from Dr. Smith of Animal Health Trust Essex, England, and 7 human phages from Dr. Nicolle of Pasteur Institute, Paris. Isolation of *E. coli* phages from faecal samples and sewage was found more dependable than Fisk's cross-culture technique. For propagation of phages heart extract broth was found superior when compared with nutrient broth and Robinson's medium.

Out of 200 strains, 59 did not show lysis by the group of 21 foreign phages. Hence 43 phages were isolated locally for typing these strains. The remaining 141 strains of *E. coli* from man and animals were grouped into 42 phage types, 12 strains being still ungroupable. Phages isolated from animal origin were seen for lysis over human *E. coli* strains and vice versa. The study gave an indication that the phage pattern of the strains of animal origin may be different from that of the strains of human origin. An observation was made to relate the maltose fermentation in the serological group of *E. coli* 055 : B5 to the prevalent phage type. Phage types present in 21 strains of this serological group in which maltose was fermented within 24 hr. were more or less similar but different from those of the strains which fermented maltose later.

While observing a difference in phage lysis between the strains isolated from normal animals and diseased, it was seen that the strains isolated from pathological conditions were not easily lysed or if at all they were the number of phages acting on them had been only one or two. Of the 67 strains of *E. coli* isolated from diseased conditions in man and animals 80% of the strains were lysed by only one or two of 64 phages in total and 20% did not show any lysis.

Fifteen strains of *E. coli* 055 : B5 obtained from cases of infantile gastro-enteritis on which 43 phages locally isolated did not show any lysis were sent to Dr. Nicolle for further phage typing. Out of this lot 9 were found to belong to St. Christopher phage type and the remaining 6 perhaps belonged to some other new type.

According to Dr. Nicolle the existence of St. Christopher phage type in India is very interesting as this is common in Great Britain, but very rare in the continent, in Germany and Hungary. Some strains have been found in Indo-china.

47. STUDIES ON THE INHIBITION OF INFECTIVITY OF PLANT VIRUSES ; S. P. RAYCHAUDHURI
INDIAN AGRICULTURAL INSTITUTE, KALIMPONG

Extracts of plant juices of chilli (*Capsicum annum* L.), spinach (*Spinacia oleracea* L.), strawberry (*Fragaria vesca* L) and datura (*Datura stramonium* L.) caused in varying degrees the inhibition of infections of potato virus X, radish mosaic and zinnia mosaic. Chilli extract completely inhibited all the three viruses while datura extract inhibited only potato virus X.

Out of seven microorganisms tested, appreciable antiviral activity was shown by the growth product of *Aspergillus niger* on radish mosaic, of *Tricothecium roseum* on radish mosaic and potato virus and of *Bacillus subtillis* on radish mosaic and vinca mosaic viruses.

Thiouracil and thio-semi-carbazide, when applied as a soil drench 2 days before and after inoculation, produced marked inhibition of potato virus X.

48. PHAGE PATTERN OF *STAPHYLOCOCCUS AUREUS* ; B. GHOSH RAY AND S. C. PAL, DEPARTMENT OF BACTERIOLOGY, ALL INDIA INSTITUTE OF MEDICAL SCIENCES, NEW DELHI

As a preliminary to the epidemiological studies of staphylococcal infections 227 strains isolated from various hospital samples were analysed for their phage pattern using standard phages obtained from the Public Health Laboratory, Colindale. Sixty per cent strains were lysed by one or more phages and their relative distribution in group I, II, III and IV were 30·5, 9, 58·5 and 2% respectively. The prevalence of phage types of strains isolated from different sources and their correlation to the antibiotic-resistance are discussed.

49. CHOLERA BACTERIOPHAGE IN PREVENTING THE GUT-INFLAMMATORY ACTION OF *VIBRIO CHOLERAE* ; S. MUKERJEE AND S. N. GHOSH, DIVISION OF MICROBIOLOGY, INDIAN INSTITUTE FOR BIOCHEMISTRY AND EXPERIMENTAL MEDICINE, CALCUTTA

Marked inflammatory reactions, characterized by intense congestion and balooning with collection of serus or sero-sanguineous fluid take place as the result of inoculation of *Vibrio cholerae* in the ligated loops of small intestine of rabbits or guineapigs. The possibilities of preventing this reaction by the use of a cholera bacteriophage were tested.

In each of a series of eight rabbits two or three loops of small intestine (4″-6″ in length) were tied at the interval of about 8 inches. Half ml. of (1 in 10 dil) 2 hr. broth culture of a recently isolated smooth strain of *V. cholerae* and the same quantity of a Group IV phage (undiluted and in progressive ten-fold dilutions) were mixed and injected immediately into the loops of guts slowly.

Inhibition of the typical reaction was observed. Reaction was totally absent in the loops receiving undiluted or lower dilutions of the phage. Segments having injections of higher phage dilutions (1,000 or above) showed some congestion but there were very little fluid collections. The few *V. cholerae* isolated from the loops were found resistant to the phage.

The results of the experiments lend some support to the case for a renewed interest in the therapeutic uses of bacteriophages in cholera as recently made out by the Russian workers.

Session VI

MEDICAL BACTERIOLOGY SECTION B

Chairman—Lt. Col. S. L. Kalra

50. BETA-HAEMOLYTIC STREPTOCOCCI IN SURVEY THROAT CULTURES; RUTH. M. MEYERS AND GRACE KOSHI, DEPARTMENT OF MICROBIOLOGY, CHRISTIAN MEDICAL COLLEGE AND HOSPITAL, VELLORE

Since the interest in the subject of correlation of the incidence of rheumatic fever and rheumatic heart disease with streptococcal infection continue to be great, it seemed worthwhile to undertake a study of these in an Indian setting. It was decided to begin with a survey to discover something of the prevalence of beta-haemolytic streptococci in cultures of throat swab specimens and the antigenic groups which might be present. For analysis specimens were classified according to the age group, or social group of the subjects concerned *e.g.*, medical students, town college students, pediatrics clinic children, village school children. Findings are presented with a comparative analysis of similar studies reported in the literature.

According to some workers, gross carrier rates for beta-haemolytic streptococci were found in Vellore as elsewhere, to be above 50% in some of the groups studied, with peak incidence occurring in the 6 to 15 year old subjects.

The pattern of antigenic groups of the beta-haemolytic streptococci isolated was found to be quite different, however, from that reported for studies conducted elsewhere. Group G strains rather than group A were found to predominate. Findings with regard to incidence of group B and group C strains also differed showing a higher incidence of both in the cultures of the Vellore study.

51- STAPHYLO-ANTITOXIN LEVELS IN THAILAND; OSCAR FELSENFELD, SEATO CHOLERA MEDICAL RESEARCH LABORATORY, BANGKOK, THAILAND

The sera of 173 Thais afflicted with diarrhoea, 159 symptomless adults and 48 children were tested for *Staphylococcus* antitoxin using the agar gel diffusion technique of Preer. The results were compared with those obtained by examining 141 sera of newcomers to Bangkok. The agglutination method was also employed.

High anti-endotoxin values were found in about 87% of the adults and about 54%

of the children. Persons living under better hygienic conditions yielded lower titres. The agglutination tests did not show any set pattern. It is believed that the high antitoxin levels represent a response to frequent exposure to enterotoxic staphylococci. This also could explain the scarcity of staphylococcal food poisoning in adults belonging to the low-income group in Thailand.

52. ON THE GENUS *CRYPTOCOCCUS KUTZING* EMEND. VUILLEMIN IN INDIA ; R. S. SANDHU D. K. SANDHU, H. S. RANDHAWA AND S. C. CHAKRAVARTY, VALLABHAI PATEL CHEST INSTITUTE, UNIVERSITY OF DELHI, DELHI

Information about the occurrence of the genus cryptococcus in India is scanty to-date. The present report describes in detail the morphological and physiological characteristics of seven isolates of cryptococcus, 5 representing *C. diffluens* and one isolate each representing *C. albidus* and *C. laurentii*. All these isolates have been cultured from human sources, five from sputum, and one each from throat swab and skin scrapings. The three species reported here are believed to be new records for India.

53. AN INFECTION DUE TO *DIPLOCOCCUS CRASSUS* ; H. N. DUTTA, ARMED FORCES MEDICAL COLLEGE, POONA

A young male child aged 6 years complained of slight fever, pain and itching sensation over the anterior portion of the penis. On examination, it was found to be a mild urethritis. There was no history of trauma or exposure as could be ascertained from the parents. Urine showed a trace of albumin, pus-cells and epithelial cells. Wassermann reaction was negative. Smear showed both Gram-positive and Gram-negative plump diplococci both intra-and extra-cellular. They looked unlike Gonococcus. The organism was isolated on blood agar under reduced oxygen tension and its biochemical and other characteristics revealed it to be *Diplococcus crassus* (*Neisseria crassus*) and not Gonococcus.

54. THE MICROORGANISMS OF URINARY TRACT INFECTIONS AND THEIR ANTIBIOTIC SENSITIVITY. ; B. N. JOSHI, J. M. MOSES, AND R. K. GADGIL, DEPARTMENT OF PATHOLOGY AND BACTERIOLOGY, GRANT MEDICAL COLLEGE, BOMBAY

The incidence of microorganisms isolated from 151 positive urine cultures is presented. The importance of Gram-negative bacilli in these infections is stressed.

Results of the antibiotic sensitivity tests conducted on the Gram-negative isolates are presented. A fairly high degree of resistance of such organisms to some commonly used antibiotics is noted.

Possible source of such Gram-negative infections is discussed.

55. GRAM-NEGATIVE BACILLI CAUSING URINARY TRCT INFECTIONS ; VIDYA RATTAN, S. N. SAXENA AND BALBIR SINGH, MAULANA AZAD MEDICAL COLLEGE, NEW DELHI

Gram-negative bacilli isolated from 300 cases of urinary tract infection were investigated for their biochemical reaction with a view to identifying the species. Correlation of clinical condition of the patients with the organism isolated from their urine was also studied.

56. A STUDY OF THE FUNGI ISOLATED FROM CASES OF OTOMYCOSIS ; L. M. MOHAPATRA, DEPARTMENT OF BACTERIOLOGY, ALL INDIA INSTITUTE OF MEDICAL SCIENCES, NEW DELHI

Otomycosis, also known as Singapore ear, hot weather ear etc. implies any fungus disease of the ear, the fungus being either the actual cause or merely a secondary invader. The fungi said to cause Otomycosis are numerous. An attempt has been made in the present study to isolate and identify the fungus from clinically diagnosed cases of otomycosis. In the present series aspergilli, penicillia, candida and a few other species have been isolated from these cases, the *Aspergilli* constituting the majority (over 90%).

The main problem in otomycosis i.e. the part played by the fungus in the morbid process is being studied in close collaboration with E.N.T. department from which source the material is collected.

57. THE ANTIGENIC ANALYSIS OE *CLOSTRIDIUM WELCHII* CULTURE FILTRATE AND COMMON ANTIGENS WITH OTHER CLOSTRIDIA ; NILIMA KAUSHIK AND S. S. RAO, HAFFKINE INSTITUTE, BOMBAY

With the aid of gel-diffusion and immuno-electrophoresis, the number of antigenic components in the culture filtrate of *Clostridium welchii* has been determined.

The toxin, lecithinase, phosphodiesterase, protease and lipase were identified in the agar electrophoresis strip using micro-procedures. The toxin and lecithinase were found to be located at the same spot. The lethal component of *C. welchii* culture filtrate has been reported to be phospholipase C. The purification of this enzyme was therefore, attempted. The toxin was fractionated on the anion exchanger DEAE cellulose using gradient elution technique into 9 components. The lecithinase was recovered in one single fraction. This fraction also had protease activity. The cation exchanger CM cellulose was used to refractionate the components not absorbed on DEAE cellulose column. Eight components were obtained, none of which appeared to have any phospholipase C or proteolytic activity.

The common antigens between *C. welchii*, and *C. oedematiens*, and *C. tetanii*, were determined by using gel-diffusion technique. Out of 6 components in *C. welchii*, one was common to *C. tetanii* and *C. oedematiens*.

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TYPING OF *ESCHERICHIA COLI* STRAINS OF ANIMAL AND HUMAN ORIGIN BY BACTERIOPHAGE

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From the Department of Pathology and Bacteriology, Veterinary College, Mathura

(Received for publication, March 1961)

Smith and Crabb (1956) described a bacteriophage method of classifying strains of *Escherichia coli* inhabiting the alimentary tract of cattle. They have divided the strains into a large number of types. The present work deals with the typing of *E. coli* strains of animal and human origin by bacteriophage.

MATERIALS AND METHODS

Faecal specimens or rectal swabs of healthy calves, buffaloe calves, lambs, goats and humans were mainly used for the isolation of strains of *E. coli*. Strains were also isolated from animals and humans showing pathological conditions: mastitis in cattle, pyelonephritis in bovines, gall bladder of slaughtered goats, vagina of cows positive for brucellosis, cases of human appendicitis and infections of the intestinal tract after operation. Out of 500 strains isolated, only 151 were used in this work. Forty-nine strains of serological group 055: B5 associated with infantile diarrhoea, obtained from Dr. (Miss) Prema Prabhu Bhat, Christian Medical College and Hospital, Vellore, were also employed.

The methods of Fisk (1942) aod Smith and Crabb (1956) were used for the isolation of bacteriophages. As pointed out by Smith and Crabb (1956), the former method was found quite unsatisfactory. A total of 34 phages were isolated locally, 18 from buffaloes and buffaloe calves, 15 from cows and calves, 2 from lambs, 4 from human and 4 from sewage. Thirteen phages (A, B, E, H, L, O, T, Z_2, Z_4, Z_5, Z_6, Z_7 and Z_8) were obtained from Dr. Smith, Animal Health Trust, Lillystone Hall Stock, Essex, England, seven (5, 7, 11, 17, 19, 20 and 22) from Dr. Nicolle, Pasteur Institute, Paris and one Cvx-5 from Dr. Gupta, Central Drug Research Institute, Lucknow.

The phages were purified by the method of Smith and Crabb (1956). Among the three media used, heart extract broth, nutrient broth and Robinson's medium, the first gave best results both for the isolation and cultivation of the phages.

The techniques of Smith and Crabb (1956) were followed for finding R.T.D., phage-differentiation and phage-typing of bacteria.

Fifty strains of *E. coli* (30 strains from normal animals and 20 from pathological conditions), isolated locally, were employed for pathogenicity test in mice. 0·1 ml.

of 18 hr. old broth culture, of Brown's opacity 6, was injected into each of a batch of three mice (Swiss strains) intra-peritonially. Observations were made up to six days and then the animals sacrificed for isolating the bacteria.

Results

Forty-three locally isolated and 21 other phages (named F phages), obtained from other places, were tested on 33 standard serological groups of *E. coli* obtained from Dr. Ørskov, Serum Institute, Copenhagen and Dr. Rees, Royal Veterinary College, London. Serological groups (081 : B1, RVC 3542 and RVC 3961) could be typed by local phages but not by F phages. The reverse was the case with the group RVC 1401.

The lytic action of F phages was studied on 200 strains of *E. coli* (see Materials and Methods). Fifty-nine strains (26 from bovines, 10 from buffaloes and buffaloe calves, 3 from goats and 20 from humans) were not lysed but the remaining strains were grouped into 42 phages types. When the unlysed strains were tested on local phages, it was observed that 15 phages from bovines caused lysis of 21 strains from bovines, 8 from buffaloe and buffaloe calves and all the three from goats; 18 phages from buffaloe and buffaloe calves lysed 23 strains from bovines, 7 from buffaloe and buffaloe calves and all the 3 from goats; 2 phages from lambs typed 7 strains from bovines and none from buffaloe and buffaloe calves and goats.

Forty-nine *E. coli* strains of human origin (serological group 055 : B5) were tested against the local phages. Fifteen of the strains were not lysed. They were sent to Dr. Nicolle for typing and the results are presented in Table I.

TABLE I

Typing of strains of E. coli *by bacteriophage*

E. coli strains	Agglutination with 055:B5 serum	d' A and C reaction*	Phage type
T I 3821	+	—	Untypable
T I 4886	+	—	Untypable, different from T I 3821
T I 5105	+	—	Untypable, like T I 4886
T I 4009	+	—	Untypable, different from T I 3821 and T I 4886
T I 5036	+	—	Untypable
T II 1141	+	+	St. Christopher 055: B5: H2
T II 843	+	+	,,
T II 1512	+	+	,,
T II 2092	+	+	,,
T II 890	+	+	,,
T II 2011	+	+	,,
T II 2118	+	+	,,
T II 1611	+	+	,,
T II 2269	+	+	,,
O 3278	+	—	Untypable, different from other untypable strains

* d' Allessandro and Comes reaction with beta phenylpropionic acid.

Among a total of 62 human strains of *E. coli*, isolated locally and belonging to different serological groups, 60% could not be typed by local phages from buffaloes, 59·8% by bovine phages and 77% by lamb phages. Smiths phages from animal sources caused lysis of only 16% of the strains, Dr. Nicolle's phages from human sources showed lysis on 66% of these strains and Dr. Gupta's phage of human origin from a patient of colitis did not attack any one of the strains. When 79 strains of *E. coli*, isolated locally from animal sources, were tested against Smith's phages and they were all lysed. Nicolle's and Gupta's phages attacked 35% and 6% of the strains respectively.

Twenty-one strains of *E. coli* (serological group 055: B5) that fermented maltose in 24 hr. and were sensitive to tetracycline group of drugs, chloramphenicol, streptomycin and neomycin could be grouped into 3 main phage types. The other strains in group 055: B5, fermenting maltose after 24 hr. or giving variable reactions and sensitive to neomycin, belonged to other phage types.

Thirty-six per cent. of the strains of *E. coli* from normal animals, and 85% of the strains from pathological cases, caused mortality of mice.

Discussion

The results of the present study show that strains of *E. coli* could be classified into phage types as pointed out by Wilson and Atkinson (1945), Smith (1948 a, b), Nicolle, LeMinor, Buttiaux and Ducrest (1952), Tee (1955) and Smith and Grabb (1956) for strains of *E. coli*, *Staphylococcus* and *Shigella*.

As pointed out by Nicolle (1960), it is interesting to note that St. Christopher's phage type seem to be common in India and Great Britain while they are rare in Germany and Hungary.

Smith and Crabb (1956) found that with phages isolated from bovines, only 17 out of 50 strains of *E. coli* from human could be typed. In the present work, we found a few phage types that were present both in human and animals. As shown by Smith and Crabb (1956), phages isolated from bovine caused lysis of strains of *E. coli* isolated from buffaloes and vice versa.

Summary

Bacteriophage typing of 200 strains of *Escherichia coli* of human and animal origin was done with 43 phages isolated locally and 21 obtained from other places. In general, the strains of *E. coli* from animal sources belonged to different phage types than the strains of human origin.

Acknowledgment

The authors gratefully acknowledge the help rendered by various workers, Dr. P. Nicolle, Pasteur Institute, Paris for supplying the phages and for typing strains of *E. coli* sent to him; Dr. H. W. Smith, Animal Health Trust, Huntingdon, England, for supplying standard phages; Dr. B. Mukerji, Director, Central Drug Research Institute, Lucknow for permission to learn the phage-typing technique and Dr. B. M. Gupta and Dr. B. N. Singh of the same Institute for proper guidance and supplying Cvx-5 phage;

Dr. (Miss) M. A. Burchard, Superintendent, Creighton Freeman Christian Hospital, Vrindaban for kind permission to collect human rectal swabs; Dr. (Miss) Prema Prabhu Bhat of Christian Medical College and Hospital, Vellore for supplying strains from infantile gastro-enteritis cases; Dr. Frits Ørskov of International Salmonella and Escherichia centre Denmark and Dr. T. A. Rees, St. George Hospital Medical School, London for supplying standard serological strains, Dr. J. B. Shrivastav, Director, Central Research Institute, Kasauli and Miss Betty A. Hughes of Royal Veterinary College, London for sending a few serotypes and strains; Dr. P. J. Glantz of Pennsylvania State University for valuable suggestions.

Thanks are also due to Major C. V. G. Choudary, Principal, U. P. Veterinary College, Mathura for providing necessary facilities.

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JOURNAL OF
MICROBIOLOGY
Vol. I, No. 2, 1961

INCIDENCE OF GRANULAR VULVOVAGINITIS IN RUMINANTS

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(Received for publication, December 1960)

Isepponi (1887) was the first to describe vulvovaginitis, affecting cattle. Its prevalence has been reported from different parts of the world. The literature has been reviewed by Edwards (1943), Durrel (1949) and Hunter, Henderson and Dardiri (1958). Polding and Lall (1945) have not mentioned the incidence of vulvovaginitis in their work on the causes of infertility in Indian cattle. Bhattacharya, Luktuke, Rao and De (1954) found vaginitis in 11·60% of buffalo cows and 5% of buffalo heifers out of 1020 genital organs examined.

Several organisms have been incriminated from the time Ostertag (1901, cited by Hunter *et al.*, 1958) isolated a *Streptococcus* and claimed to have reproduced the disease with his isolate. Subsequent workers (see Durrel, 1949; Brion, 1951, cited by Hunter *et al.*), however, could not confirm Ostertag's findings. Troise and Guida (1956) reported that a haemolytic *Streptococcus*, isolated from infected animals, could

reproduce the disease. A variety of other organisms have also been isolated, a gram-negative, bipolar, microaerophilic bacillus by Jones and Little (1927) and Ullrey (1951, cited by Hunter *et al.*, 1958), a *Haemophilus* by Crawley, Wills and McGregor (1950) and Cockman (1950, cited by Hunter *et al.*, 1958) and it has been claimed that the reproduction of the disease with these organisms is possible. Blaha (1909, cited by Hunter *et al.*, 1958) suggested protozoal parasite as the causative agent. McIntosh, Haigh and Alexander (1954), Kendrick, McKercher and Saito (1956) and McClure (1957) have supplied evidence of virus being the aetiological agent.

Materials and Methods

Gross pathological examinations were conducted in ruminants for the detection of the disease by examination of vulva and vagina in strong day light and lesions, when present, were graded as severe, mild and very mild as outlined by Hunter *et al.* (1958). Altogether 587 animals (399 cattle, 73 buffaloes, 100 goats and 15 sheep) have been examined for the presence of granular vulvovaginitis.

Results

From Table I, it would appear that in cattle, with three different breeds, the incidence of granular vulvovaginitis was high, ranging from 82·9% to 100·0%. A correlation of the incidence with age groups would show that there is little difference between incidence rate among cows, heifers and calves. In all breeds, irrespective of age, majority of cases were of severe type. In buffaloes, the cases of the severe type of the disease were much less than in cattle. Similarly, in goats, the incidence of severe cases was low. In sheep it was 100%. As the number of sheep examined was only 15, it would be of interest to examine more animals for the prevalence of the disease. It was also found that pyaemic infection, resulting in varying degrees of purulent discharge, occurred in some of the animals suffering from granular vulvovaginitis. This was maximum in goats (35·0%), followed by cattle (7·8%) and buffaloes (5·0%). In cattle, the incidence was highest in calves decreasing gradually with the increase of age, while in buffaloes the position was reversed.

Bacteriological examinations of the vagina of a number of affected and healthy cows and buffaloes did not result in the isolation of any specific bacteria that could be incriminated as the causative agent of the disease.

Discussion

Williams (1947) and Gibbons and Clark (1956) have considered granular vulvovaginitis in cows as a herd problem. In view of the very high incidence in cattle and other ruminants examined, the authors are inclined to consider this disease of gynaecological interest. The incidence, however, appears to vary from place to place in view of the observations of Bhattacharya *et al.* (1954), who recorded an incidence rate of 5·0 to 11·69% in buffaloes as against 44·4 to 66·6% observed in the present study. It will be of considerable interest to know more about the incidence in other parts of India, specially in cattle.

TABLE I

Incidence of vulvovaginitis in ruminants

Breed	Age Group	No. of animals examined	No. of positive cases	Severity in positive cases		
				Severe %	Mild %	Very mild %
			Cattle			
Red Sindhi	Cows	90	79	68·0	16·5	15·5
	Heifers	36	34	86·5	13·5	Nil
	Calves 4 months to 1 year	37	35	83·0	14·0	3·0
Hariana	Cows	22	22	81·8	18·2	Nil
	Calves 4 months to 1 year	12	11	72·7	27·3	Nil
Indigenous	Cows	106	92	57·0	32·0	11·0
	Heifers	20	18	72·0	22·0	6·0
	Calves upto 1 year	76	63	54·0	36·5	9·5
		399	354			
			Buffaloes			
Murrah	Cows	45	30	28·1	29·2	42·7
	Calves	28	13	38·6	30·7	30·7
			Goats			
Beetal	Adult	12	4	50·0	225·0	25·0
Cross-Beetal	Adult	88	20	30·0	60·0	10·0
			Sheep			
Cross Banur	Ewes	15	15	100·0	Nil	Nil

The incidence of granular vulvovaginitis in cattle, buffaloes, sheep and goats has been recorded for the first time in Orissa. Probably, this is also the first authentic record of this disease in different species of ruminants, other than buffaloes, in India.

Summary

The incidence of granular vulvovaginitis was high (82·9—100%) in cattle. There was little difference in the incidence rate with age groups among cows, heifers and calves. Most cases were of severe type. In buffaloes and in goats the incidence of severe cases was low, while in sheep it was 100%. Bacteriological examination of the vagina of affected animals did not reveal the causative agent of the disease.

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LETTER TO THE EDITOR

SOME OBSERVATIONS ON THE BEHAVIOUR AND COLOUR OF *PARAMECIUM MULTIMICRONUCLEATUM* POWERS AND MITCHELL, 1910

When a sample of water is collected and examined for its microscopic fauna particularly protozoa, it is generally found that it is not infested with only one type of organism (if any at all); but instead, it is commonly found to be a heterogeneous collection of animals. It is thus a problem to isolate a particular type of organism for experimental purposes. A very simple method for isolating paramecia from such a heterogeneous collection was accidentally found out in our attempt to isolate a free living amoeba.

A few drops of water containing the material (different types of organism) was taken in a petridish containing Chalkley's medium (1940) in which a few grains of rice (3 or 4) were then scattered. After 2 to 3 days it was found that each rice grain had developed a whitish slime around it which was thickly populated with paramecia (fig. 1). On removal of the rice grain its under surface was also found to be thickly populated with the ciliate. It appeared that the organisms were more or less entangled in the slime and did not easily scatter away unless disturbed mechanically or by strong light. Apparently the organisms found a rich source of nutritive material in the slime surrounding the rice. Or primarily, the crowding of paramecia round the rice particle might have been due to the production of fermentative CO_2 at the site, as pointed out by Jennings (1931) and, later, the ciliate stuck to that richly nutritive environment. These paramecia could then be easily taken out by means of a pipette and cultured in hay infusion or in any other common culture media. With further disintegration of the rice, *i.e.*, on the 4th or 5th day, the population of *Paramecium* became reduced on the whole and when the hydrogen ion concentration dropped down (*p*H 4), the organisms were all dead. Amoebae though survived for a short while, the media never showed a very rich concentration of them in any particular area. The experiments were repeated several times with the same result. It may not be irrelevant to mention here that this method is being employed for a long time to isolate amoeba and has almost become classical in amoeba culture. The most interesting point to note here is that the original culture though contained a species of amoeba, it was the ciliate which dominated round the rice grain and not the amoeba.

Fig. 1.

Another interesting point observed was that the same strain of paramecia (*P. multimicronucleatum*), which appears yellowish or brownish grey and opaque in hay infusion became white and rather transparent when put in Chalkley's medium and vice versa. In this context it may be mentioned that change of colour with the change of medium has been recorded earlier in *P. woodruffi* —a brackish water form. There it was shown that the normal pale yellow colour of the organisms disappeared when put in fresh water and addition of a small amount of sea water in the fresh water culture resulted in partial restoration of the yellow colour. No such phenomenon, has yet been recorded for *P. multimicronucleatum*. According to Wichterman (1953) *P. caudatum* was either light grey or white in colour but nothing has been stated as to whether the colour of the animal had any relation to the medium.

Department of Protozoology,
School of Tropical Medicine,
Calcutta-12. India.
December 1, 1960.

H. N. Ray
and
B. Hajra

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ASSOCIATION NEWS

FOURTH ANNUAL GENERAL MEETING OF THE ASSOCIATION OF MICROBIOLOGISTS OF INDIA AT DELHI

The 4th Annual General Meeting of the Association was held in the Old Convocation Hall, University of Delhi at 11 A. M. on 2nd April, 1961. Lt.-Col. S. L. Kalra presided over the meeting. The meeting transacted the following business.

1. *Adoption of Secretary's Report for* 1960

The report of the activities of the Association for 1960 was presented by the General Secretary and was unanimously adopted.

2. *Account for* 1960

The audited statement for income and expenditure of AMI was presented by General Secretary. The statement which had been adopted at the Central Council meeting on the previous day was unanimously adopted in the General Meeting. On a suggestion accepted in the Central Council meeting, it was decided that in future collections should be shown on zonal basis.

3. *Budget for* 1961.

The proposed budget of the Association was presented and explained by the General Secretary. Certain clarifications were sought for by members which were explained by him. The budget was unanimously adopted.

It was then resolved that for the sake of convenience in compilation, auditing and circulation of the account before the Annual General Meeting, the financial year of the Association be taken as between 1st November of one year to 31st October of the next year.

The suggestion to utilise some advertising agency for collecting advertisement for the AMI journal on behalf of the Association was noted for future action.

4. *Election of the Central Council for* 1961

The General body meeting unanimously accepted the recommendation of the Central Council and elected the following members for the Central Council.

President:	Dr. J. C. Ray—Calcutta
Immediate Past President:	Major General S. S. Sokhey—New Delhi
Vice Presidents:	Dr. P. V. Gharpure—Bombay
	Dr. M. N. Lahiri—Calcutta
	Dr. S. Govindarajan—Madras
	Dr. B. N. Singh—Lucknow

General Secretary: Dr. S. Mukerjee—Calcutta

Additional Secretaries: Dr. D. D. Banker—Bombay
Dr. (Miss) Ruth M. Meyers—Vellore
Dr. B. M. Gupta—Lucknow
Dr. A. Narayanaswami—Calcutta

Treasurer: Dr. M. S. Das—Calcutta

Editor of AMI *Journal:* Dr. B. N. Singh—Lucknow

Representative to the International Association of Microbiological Societies—
Dr. S. Mukerjee (*General Secretary*)

The following persons were elected as representatives of the zonal units and general members:—

Northern Zone	Dr. B. Ghosh Ray—New Delhi
	Dr. C. M. Singh—Mathura
	Dr. R. N. Singh—Varanasi
Eastern Zone	Dr. A. N. Roy—Calcutta
	Dr. M. K. Mukherjee—Barrackpore
	Dr. S. C. Seal—New Delhi
Southern Zone	Dr. A. Sankaram—Bapatla
	Dr. M. N. Pai—Madras
	Dr. R. Ananthanarayanan—Trivandrum
Western Zone	Dr. H. S. Andleg—Bikanir
	Dr. F. Fernedes—Bombay
	Dr. H. I. Jhala—Bombay
General (irrespective of zones)	Lt.-Col. S. Datta—Calcutta
	Dr. (Miss) Y. M. Freitas—Bombay
	Dr. T. S. Sadasivan—Madras
	Dr. W. V. B. Sundara Rao—New Delhi

It was resolved that Dr. M. S. Das, Principal, Bengal Veterinary College, Calcutta, who has been elected as Treasurer of AMI for 1961 in place of Dr. S. C. Seal, will operate the Bank account of the Association of Microbiologists of India jointly with Dr. S. Mukerjee, the Secretary.

The meeting further resolved that as the strength of membership of the Association has gone up it is necessary to increase the number of the representatives in the Council. It was resolved that General Secretary may take necessary steps to get the constitution

suitably amended so as to make provision for 4 representatives in place of 3 from each zonal unit and 8 members in place of 4 as the general representatives, irrespective of zone.

5. *Appointment of the honorary auditor and the honorary accountant for* 1961

The general meeting of AMI recorded their appreciations for the honorary services rendered by Shri N. N. Banerjee, Chartered Accountant, Calcutta as the Auditor and Shri D. Chatterjee, Calcutta as an Accountant for the year 1960. The meeting re-elected Shri N. N. Banerjee as the honorary auditor and Shri D. Chatterjee as the honorary accountant of AMI for 1961.

6. *Representatives to the International Microbiological Congress at Montreal*

The meeting approved the authorization of the President of the AMI to select the suitable candidates to represent the Association in the International Microbiological Congress at Montreal in the year 1962.

7. *Distribution of the Journal of* AMI

The General meeting also approved the decision of the Central Council that the first copy of the Journal of AMI be sent to all members of the Association, whether they have paid their subscription up to date or not. It was further decided that a request should be made to the members to let the office know if further copies of the journals are required by them. In each of the cases arrear membership subscription has to be paid up to date. Failing this the second copy is to be sent by V.P.P.

8. *Venue of next session of* AMI

The recommendation of the Central Council regarding the venue of the 5th congress and the invitation from the Christian Medical College, Vellore, for holding the next AMI Session there were discussed. It was decided that the Annual Session of the Association may preferably be arranged at Madras. The programme of one of the days may be organized at Vellore. The southern zonal council was authorised to make the final choice of the place.

It was also decided that the 6th Annual Congress of AMI will be held in Calcutta.

9. *The teaching sub-committee of* AMI

In the group discussion of "Teaching of Microbiology in the Indian Universities," it was recommended that the Central Council should form a Sub-committee for implementing the constructive ideas and suggestions that have come out in the discussion. The General Body members appreciated the importance of taking active steps for giving shape to the constructive ideas. A Sub-committee with the following members was formed with powers to co-opt new members:

Dr. J. C. Ray—Calcutta	*President*
Dr. J. B. Shrivastav—Kasauli	*Vice-President*

Dr. B. M. Gupta—Lucknow	*Secretary*
Dr. P. V. Gharpure—Bombay	*Member*
Dr. S. L. Bhatia—New Delhi	,,
Dr. Y. Freitas—Bombay	,,
Dr. S. C. Seal—New Delhi	,,
Dr. Lakshmi N. Rao—Ludhiana	,,
Dr. C. M. Singh—Mathura	,,
Dr. M. S. Das—Calcutta	,,
Dr. R. N. Singh—Banaras	,,
Dr. W. V. B. Sundara Rao—New Delhi	,,
Dr. B. Ghosh Ray—New Delhi	,,
Dr. R. Ananthanarayanan—Trivandrum	,,
Dr. T. S. Sadasivan—Madras	,,
Dr. S. N. Dasgupta—Kalyani	,,
Dr. R. S. Vasudeva—New Delhi	,,

INSTRUCTIONS TO CONTRIBUTORS

It is the editorial policy of this Journal to publish papers on original research in microbiology in its widest aspect, *i.e.*, the study of viruses, bacteria, microfungi, microscopic algae and protozoa and, particularly, the fundamental aspects of the study of these micro-organisms. The Editorial Board will consider only original material for publication. Once a paper is accepted for publication it should not be published elsewhere either in English or in any other language without the consent of the Editor.

Correspondence relating to the Journal and papers for publication may be addressed to Dr. B. N. Singh, Editor, Indian Journal of Microbiology, Central Drug Research Institute, Lucknow, India.

The Journal will be issued quarterly one volume appearing in a year. The Journal will also publish Letters to the Editor and reviews.

Manuscripts may be communicated in typescript in a final and finished state with double line spacing and ample margins. A paper may in general be divided into the following parts (*a*) Introductory paragraph; (*b*) Materials and methods; (*c*) Results; (*d*) Discussion; (*e*) Summary; (*f*) Acknowledgments (if any) and (*g*) References.

Two copies of each manuscript should be submitted. A short running title, suitable for page-headings, should be furnished. The name of the laboratory where the work was done should be indicated on the title page.

Authors are responsible for preparing a paper in a form suitable for sending to press. Careful preparation of manuscript will make for prompt publication.

Illustrations may, if possible, be drawn on Bristol board in Indian ink with lettering inserted lightly in pencil. Author's name, short title of the paper, fig. no. etc. should be marked at the back of the illustration. Drawings may be larger than the size of the printed block, and their order and approximate position in the text should be marked. Line drawings will be referred to as Figure 1, Figure 2, etc and half-tone blocks as Plate I, Plate II, etc. Besides the original illustrations one duplicate set must accompany the second copy of the manuscript.

Tabular matter may be kept to a minimum.

Spelling should conform to current English usage according to the Concise Oxford Dictionary.

Binomial Latin names of micro-organisms should be given in full when first mentioned in a paper and subsequently with the generic name abbreviated. They should be underlined in the typescript.

The following symbols and abbreviations may be written in the manner shown: degrees Centigrade are written, *e.g.* 100°; hr., min., sec. (singular and plural); *M*, Molar; *N*, normal (of solutions); m, milli-(10^{-3}) and, micro-(10^{-6}); *e.g.*, ml., millilitre (instead of cc.) and μg. (instead of γ), microgram; No. or no., number; dillutions should be written 1/10.

References should be arranged in the alphabetical order of the authors' names with abbreviations according to the World List of Scientific Periodicals. Initials of the authors' forenames should be given, but not the title of the paper. For example, Cutler, D. W., Crump. L. M. and Sandon, H. (1922) *Phil. Trans.* B, **211**, 317.

References to books and monographs should include the town of publication and the name of the edition to which reference is made. The arrangement should be as in the following example: Kudo, R. R. (1964) *Protozoology*, 4tz edition, Charles C. Thomas, Springfield, Illinois, U.S.A.

Citations in the text should read thus: (Bose, Ghose and Roy, 1950). When a paper has more than two authors, the names of all of them should be given at its first citation in the text (unless there are more than five authors) and in subsequent citations thus: (Bose *et al.*, 1959). The conventions Bose, (1959a), Bose, (1959b) should be used where more than one paper by the same authors has appeared in one year.

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INDIAN JOURNAL OF MICROBIOLOGY

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Vol. I, No. 2, 1961.

PRINTED AT SREE SARASWATY PRESS LTD., 32, ACHARYA PRAFULLA CHANDRA ROAD, CALCUTTA-9, INDIA